Adiposity and the Brain

The adiposity-brain-axis in mice and men

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‘Omdat je altijd moet doen wat je leuk vindt’

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GENERAL INTRODUCTION

Parts of the general introduction are based on:
Adipokines: a link between obesity and dementia? Kiliaan A.J., Arnoldussen I.A.C. and Gustafson D.R. (2014). The Lancet Neurology 13(9): 913-923.

Obesity and dementia: adipokines interact with the brain. Arnoldussen I.A.C., Kiliaan A.J., and Gustafson D.R. (2014). European Neuropsychopharmacol 24 (12): 1982-99.

Impact of DHA on metabolic diseases from womb to tomb. Arnoldussen I.A.C. and Kiliaan A.J. (2014). Marine Drugs 12 (12):6190-212.

General introduction

Worldwide, obesity has reached epidemic proportions, and each year obesity or obesity-related-diseases lead to the death of 2.8 million adults (1). Obesity has an undefined aetiology, but is generally caused by imbalance of energy intake versus energy output. This imbalance results in excessive, mainly white adipose tissue (WAT) accumulation, adiposity. Adiposity may eventually have an adverse effect on human health, which can evolve in adiposity related diseases such as cardiovascular disease, type 2 diabetes mellitus (T2DM) and even cognitive impairment and dementia (1-3). Individuals are considered obese when their body mass index (BMI) exceeds 30.0 kg/m^2. BMI grossly reflects total WAT during adulthood (4). Although, particularly in older individuals, BMI may not be an accurate marker as aging is characterized by lean body mass loss and WAT increase without gaining weight, a phenomenon that is not captured by BMI (5). In addition, the amount of visceral adipose tissue is associated with higher waist circumference (WC) or waist hip ratio (WHR), and therefore this has been another rationale for determining central body adiposity (6-8).

Fatty acids

Intake of diets with a high content of saturated fat (HFD) or high fat and high carbohydrates (HFHC) are considered to be important contributing factors of adiposity. Moreover, high intake of saturated fatty acids is associated with development of non-alcoholic fatty liver disease, hyperlipidemia, and a low state of chronic adipose inflammation characterized by a high level of macrophages and pro-inflammatory cytokines (9-11). Hyperlipidemia, reinforced by inflammatory processes, disrupts vascular function resulting in for instance hypertension, atherosclerosis, and cardiovascular disease (12, 13). Thus, high intake of HFD and HFHC can contribute to the development of adiposity and cardiovascular disease.

On the other hand, uptake of adequate levels of saturated and essential unsaturated fatty acids from dietary sources is indispensable as they serve as building blocks for hormones and phospholipids. For instance, the long-chain polyunsaturated fatty acids (LCPUFAs), docosahexaenoic acid (DHA) and arachidonic acid (ARA) are essential components of phospholipids, can have anti-inflammatory effects, and improve vascular function. Phospholipids form the lipid bilayer of neuronal membranes therewith maintaining optimal membrane fluidity and signal transmission (Table 1)(14, 15). These LCPUFAs have potency in the prevention and treatment of adiposity and adiposity-related-diseases as for instance DHA and ARA can modulate WAT storage, reduce inflammatory processes, improve endothelial function, cerebrovascular reactivity and memory consolidation (16-19). In addition, promising results are found in examining the short chain fatty acid, butyrate as treatment for adiposity and adiposity-related-diseases (20). Butyrate

is a product of bacterial fermentation of mainly indigestible plant polysaccharides and resistant starch in the colon. Obese individuals have a decreased concentration of butyrate-producing bacteria and dietary butyrate intake may protect against diet-induced adiposity, improve insulin sensitivity and increases energy expenditure (21-24). Moreover, butyrate has anti-inflammatory properties and is involved in the regulation of liver lipogenesis, adipocyte differentiation, and energy intake (23). Thus, dietary interventions such as LCPUFAs, butyrate or even indigestible fiber rich diets, could contribute to the prevention and decline of adiposity-related-diseases and adiposity.

	Year	N	Age (Years)	DHA	Outcome
Childhood					
Desci (25)	2002	80 ♂ & ♀	12	Plasma phospholipids, ARA & DHA	Values of arachidonic acid and docosahexaenoic acid were significantly lower in diabetic children than in controls.
Burrows (26)	2011	48 ♂ & ♀	Non-obese: 9.0±0.9 / Obese: 8.9±1.2	Erythrocyte fatty acid; the Omega-3 index (O3I) composition	Obese children had altered erythrocyte fatty acid composition unrelated to reported dietary intake. A greater proportion of obese children had an omega-3 index of < 4.0 (high risk) compared with non-obese children.
Vasickova (27)	2011	120 ♂ & ♀ obese	10.0±1.9	300mg/d DHA + 42 mg/d EPA for 3weeks	Daily consumption of 300mg DHA and 42 mg EPA for three weeks leads to an improvement of the anthropometric and lipid parameters in obese children (27).
Juarez Lopez (28)	2013	201 ♂ & ♀ obese and insulin resistant	11.6±0.7	12 weeks LC-PUFA supplementation, 360mg EPA & 240 mG DHA daily	LC-PUFA supplementation for 12 weeks decreased the concentrations of glucose, insulin, triglyceride-levels and BMI.
Damsgaardt (29)	2013	73 ♂ & ♀	10.29±0.58	Plasma DHA &EPA concentrations	DHA was positively associated with mean arterial pressure in boys.
Adolescence					
Dangardt (30)	2012	25 ♂ & ♀	15.6±0.9 ♀ 15.7±1.0 ♂	1,2 g/d LC-PUFAs (DHA & EPA) for 3 months	Three months of supplementation of omega-3 LCPUFA improved glucose and insulin homeostasis in obese girls without influencing body weight.
Adulthood					
Rivellese (31)	1997	16 ♂ & ♀ NIDDM patients with hypertriglyeridemia	56.0±3.0	First two months: 0.96g EPA and 1.59 g DHA per day Last four months: 0.64 gr EPA and 1.06 gr DHA per day	DHA and EPA significantly reduced plasma triglycerides and VLDL- triglycerides without significant changes in blood glucose control.
Mori(32)	1999	56 ♂ overweight & hyperlipidemic	49.1±1.2	4 g/day DHA, EPA or olive oil (placebo) for 6 weeks	Purified DHA but not EPA reduced ambulatory BP and HR in mildly hyperlipidemic men.
Mori(33)	2000	59 ♂ overweight & hyperlipidemic	50.6±1.4	4 g/day DHA, EPA or olive oil (placebo) for 6 weeks	DHA enhances vasodilator mechanisms and attenuates constrictor responses in the forearm microcirculation.
Woodman(34)	2003	♂ & ♀ (Hypertensive and diabetic)	40-75	4 g/day DHA, EPA or olive oil (placebo) for 6 weeks	DHA increased low density lipoprotein particle size
Kelley (35)	2007	34 ♂	55.0±2.0	7.5 g DHA-oil for 90 days	DHA supplementation for 45 d significantly decreased concentrations of fasting triacylglycerol, large VLDL, and intermediate-density lipoproteins and the mean diameter of VLDL particles.
Sneddon(36)	2008	69 ♂	32.4±2.3	3 g/day CLA + 3 g/day omega-3 LC-PUFAs	Supplementation with conjugated linoleic acids (CLAs) plus omega-3 LC-PUFAs prevents increased abdominal fat mass and raises fat-free mass and adiponectin levels in obese adults
Micallef (37)	2009	124 ♂ & ♀	43.79±2.22	Plasma levels of DHA & EPA	BMI, waist circumference and hip circumference were inversely correlated with n-3 PUFA, EPA and DHA (P < 0·05 for all) in the obese group. Obese individuals had significantly lower plasma concentrations of total n-3 PUFA, compared with healthy-weight individuals.
Stirban (38)	2010	34 ♂ & ♀ (T2DM)	56.8±8.3	2 g/d EPA & DHA for 6 weeks	6 wk of supplementation with LC-PUFAs reduced the postprandial decrease in macrovascular function relative to placebo. LC-PUFAs supplementation improved postprandial microvascular function.
Itariu (17)	2012	55 ♂ & ♀ (obese)	39±2.0	3,36 g/d EPA & DHA for 8 weeks	n−3 PUFAs, which was well tolerated, decreased the gene expression of most analyzed inflammatory genes in subcutaneous adipose tissue (P < 0.05) and increased production of anti-inflammatory eicosanoids in visceral adipose tissue and subcutaneous adipose tissue (P < 0.05).
Labonte (39)	2013	12 ♂ (obese +T2DM)	54.1±7.2	3 g/d EPA & DHA for 8 weeks	In obese patients with T2DM, EPA&DHA supplementation did not affect the gene expression of pro-inflammatory cytokines in duodenal cells.
Singhal (40)	2013	328 ♂ & ♀	28.± 4.8	1,6 gr/d DHA	DHA supplementation did not improve endothelial function in healthy adolescents. Only triglyceride and very low-density lipoprotein concentrations were significant lower in DHA-supplemented individuals compared with controls.

McDonald (41)	2013	22 ♂ & ♀ Hypertensive and T2DM	58.6±8.8	Daily supplementation of 1.8 g EPA and 1.5 g DHA for 8 weeks	LC-PUFAs diminish platelet superoxide production in T2DM hypertensive patients *in vivo*.
Virtanen (42)	2013	2122 ♂	53.1±5.1	Serum levels DHA, EPA, DPA	Men with higher serum level of EPA+DHA+DPA had a 33% lower multivariate-adjusted risk for T2DM. (Trend: P=0.01)
Elderly					
Woodman(43)	2003	51 (39-♂ & 12-♀) Hypertensive and diabetic	61.2±1.2	4 g/day DHA, EPA or olive oil (placebo) for 6 weeks	DHA supplementation significantly reduced collagen aggregation and collagen-stimulated thromboxane release.
Lemaitre (44)	2003	54: Ischemic heart disease 125: non-fatal myocardial infarction	79.1±7.5	DHA & EPA plasma phospholipids	Higher combined dietary intake of DHA and EPA, and possibly α-linolenic acid, may lower the risk of fatal ischemic heart disease in older adults.
Tsitouras (45)	2008	12 ♂ & ♀	66.1±4.5	Supplemented with 4 g/day EPA and DHA	Insulin sensitivity increased significantly after 8 weeks on the EPA- and DHA-diet, and serum C-reactive protein was significantly reduced.
Heine-Böring(46)	2010	1570 686-♂ & 884-♀	64.0±5.42 - ♂ 64.0±5.6 - ♀	Food intake questionnaire; Dutch food composition table (DHA & EPA levels)	Subjects with a fish intake > 19 g/d had a significantly lower prevalence of mild/moderate calcification. EPA plus DHA intake showed no significant associations.
Djousse (47)	2011	3088 ♂ & ♀	75.0	Plasma phospholipids DHA and EPA	DHA is not associated with a higher incidence of T2DM, and individuals with higher EPA and DHA plasma concentrations had lower risk on T2DM.
Mozaffarian (48)	2011	3630 ♂ & ♀	75.0±5.0	Plasma phospholipids: EPA, DHA & DPA	Circulating individual and total omega-3 fatty acid concentrations are associated with lower incidence of congestive heart failure (CHF) in older adults.
Virtanen (49)	2012	768 396-♂ & 372-♀	60.7±6.5	Serum levels DHA, EPA, DPA	Serum EPA+DHA+DPA was associated with lower systolic blood and pulse pressure, not with diastolic blood pressure. Similar results when DHA, EPA and DPA were investigated individually.

Table 1. DHA and metabolic diseases through lifetime. *DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; CLA: conjugated linoleic acids; CHF: congestive heart failure; T2DM: type 2 diabetes mellitus; NIDDM: non-insulin-dependent diabetes mellitus; VLDL: very-low-density-lipoproteïnen; N: number of participants; Age is represented in years and in Mean±SD. Mean age >4 and ≤12 Year →childhood; Mean age >12 and ≤21→ adolescence; Mean age >21 and ≤60 → adulthood; Mean age >60 → elderly.*

Adiposity and brain function

Adiposity has been associated with alterations in brain structure and function, cognitive deficits, and even dementia and Alzheimer's disease (AD) (50-55). For instance, loss of gray and white matter integrity, functional connectivity, and cortical thickness are observed in children, adolescents and adults (52, 54, 56-62). Moreover, abnormalities in functional brain regions found in obese adults such as in the amygdala, hippocampus, and frontal cortex (63, 64). Age is crucial in examining the association between adiposity and the brain (Fig. 1 and 2) as individuals with midlife obesity have a higher risk of developing dementia in late life (65-70), whereas late life obesity seems to reduce dementia risk (71-74). In addition, adiposity may lead to damage manifested as structural changes similar to those seen in normal aging (75, 76). Exact biological pathways linking adiposity to brain processes are multi-factorial and still unrevealed (77-81). In this thesis we will discuss two pathways via which adiposity might affect brain structure, function and cognition with emphasis on the (cerebro)vascular pathway and adipokines.

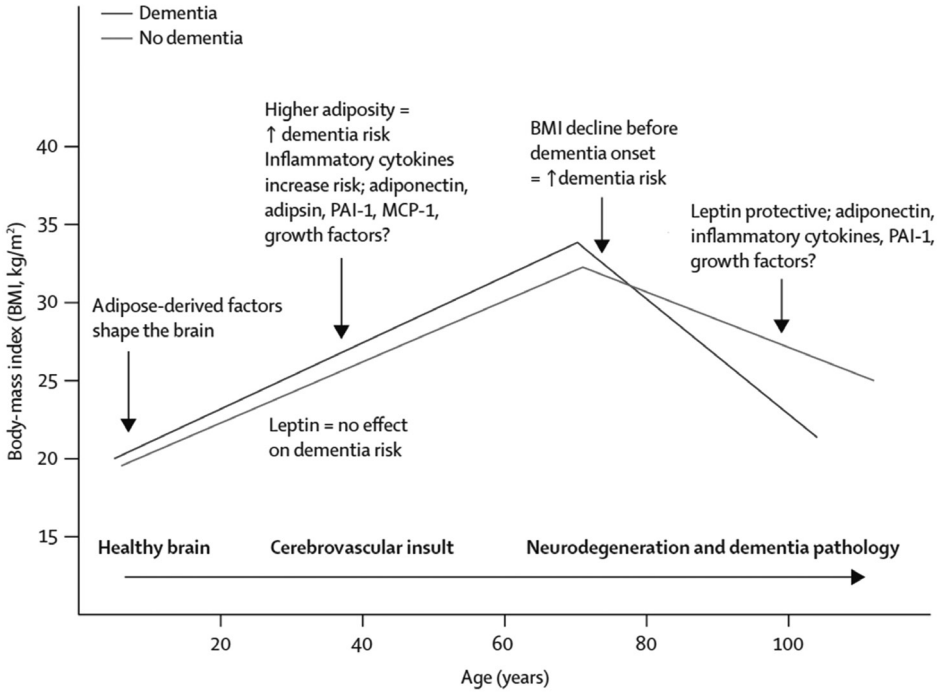

Figure 1. *Possible life course trajectory of BMI in association with dementia MCP-1=monocyte chemotactic protein-1. PAI-1=plasminogen activator inhibitor-1. Reviewed adipokines regulate: energy balance and metabolism; thrombosis and hypertension; inflammation.*

(Cerebro) vascular pathway

Subsequent to the dramatic rise of adiposity, the prevalence of insulin resistance and premature cardiovascular disease increased (82). Insulin resistance plays a central role in the pathogenesis of T2DM and develops long before the onset of the disease (83). Individuals with peripheral insulin resistance often show reduced insulin sensitivity in the brain, which can affect cognitive functioning (84, 85). Two-third of adults in the U.S. are diagnosed as overweight or obese of which 26 million have T2DM (86). Moreover, two-third of severely obese children younger than 12-years of age, develop cardiovascular risk factors (87), and obese individuals show a significantly increased risk of cardiovascular disease as well (86). Importantly, particularly clustering of adiposity, T2DM and cardiovascular risk factors has been linked to alterations in vasculature (e.g. arterial stiffness, hypertension and atherosclerosis), hypoperfusion, structural brain changes, cognitive impairment, and dementia (Fig. 1 and 2)(85, 88).

We hypothesize that adiposity can induce (cerebro)vascular changes such as arterial stiffness, inflammation and hypoperfusion, which can subsequently disrupt brain structure and function, and increase the risk for cognitive impairment and dementia. In more detail, the brain relies on perfusion as a constant cerebral blood flow (CBF) ensures constant oxygen and glucose supply and drainage of 'waste products'. In humans, overweight and obese individuals often have a decreased CBF (18). A decreased CBF, or hypoperfusion may be a result of reduced small vessel density, inflammation, arterial stiffness, atherosclerosis of the large and small vessels around/within the brain, and/or impaired vasodilation. Hypoperfusion can result in reduced gray and white matter volumes, which subsequently may evolve in cognitive impairment (18, 89, 90). Neuroimaging in obese individuals revealed structural brain changes like white and gray matter abnormalities, already at a very young age (89-92). These structural brain abnormalities may evolve in functional alternations as adiposity is associated with poorer executive functioning skills such as inhibitory control and working memory, and subsequently increases the risk to develop cognitive impairment and dementia (3, 93-95).

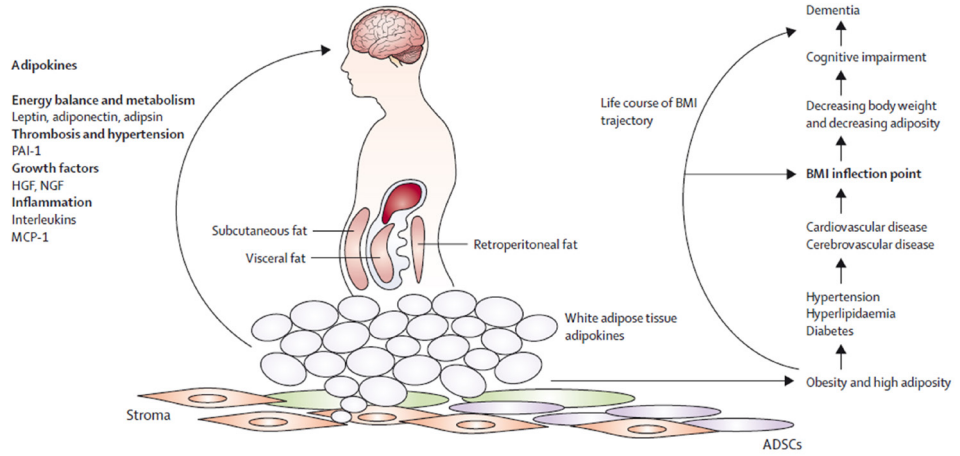

Figure 2. *The trajectory of body mass index over the life course by chronological age and potential roles of adipokines. PAI-1=plasminogen activator inhibitor-1. HGF=hepatocyte growth factor. NGF=nerve growth factor. MCP-1=monocyte chemotactic protein-1. BMI=body mass index. ADSC=adipose-derived stem cells.*

Adipokines

WAT is a complex tissue consisting of multiple cell types with multiple cellular phenotypes depending on cell composition and location (96). WAT secretes hundreds of polypeptides, adipokines, and includes cytokines, acute phase reactants, growth factors and other inflammatory mediators, adipose tissue hormones such as leptin, and other chemical messengers (51, 77, 97). The adipokines secreting profile is fat

depot-specific, which is coherent with differences in adipocyte morphology and the local milieu (98-100). Adipokines act in autocrine, paracrine, or endocrine ways (101), and many affect processes in the periphery and the central nervous system. In addition, brown, epicardial and pancreatic adipose tissue appear to have unique adipokine profiles (102-104). Adipokine release may be dysregulated in obesity, possibly because the adipose tissue may be 'diseased'. Since neurodegenerative and vascular processes observed in dementia affect several brain regions and nuclei (105), the action of adipokines might be altered during neurodegenerative and vascular events, and may even feedback to contribute to neurodegeneration.

Over 700 adipokines have been reported in response to specific chronic or acute stimuli or at rest (106). In table 2 and 3 we present adipokines and their primary known function such as metabolism (e.g. leptin, adiponectin, adipsin), vascular function (e.g. C-reactive protein, serum amyloid A, plasminogen activator inhibitor-1), and inflammatory processes (e.g. interleukins, monocyte chemotactic protein-1) (77, 88). Each may possess more than one function, and these functions do overlap (107). Figure 3 represents an overview of possible pathways of adipokines affecting the brain. Adipokines are extensively reviewed in *Kiliaan et al.* and *Arnoldussen et al.*, and therefore we will shortly discuss the most studied in humans (77,88).

Leptin, a satiety hormone regulates food intake and energy expenditure (108, 109). Observations from human population studies in late life suggest that high leptin levels and high BMI are associated with lower dementia risk when measured within 10 years of clinical dementia diagnosis (110, 111). Leptin can affect hippocampal memory formation as it enhances N-methyl-D-aspartate receptor function and subsequently enhances long term potentiation formation at hippocampal CA1 synapses and rapidly remodels dendrites (112-114). Moreover, leptin can affect vascular function by enhancing platelet aggregation and arterial thrombosis, promoting angiogenesis and inducing proliferation, and migration of vascular smooth muscle cells (115-117). Leptin seems to enhance the calcification of vascular cells, making also the arterial wall a target of leptin (117). While leptin levels may trend with BMI in relation to dementia, given the strong correlation between them, leptin may also have an independent role in health of the ageing brain.
Adiponectin modulates inflammatory responses, energy expenditure, food intake, and a number of metabolic processes including glucose regulation and fatty acid catabolism (118). Adiponectin protects plaque rupture by the inhibition of matrix metalloproteinase function (117). Moreover, endothelium-dependent vasoreactivity is impaired in people with hypoadiponectinemia, which might be at least one cause of hypertension in visceral obesity, probably by inhibiting the expression of adhesion molecules (117, 119).

Energy balance and metabolism
Adiponectin
Adipsin (complement Factor D)
Apelin
Chemerin
Dipeptidyl peptidase-4 (DPP-4) adenosine deaminase complexing protein 2 or CD26
Leptin
Lipocalin
Omentin
Resistin
Retinol binding protein-4
Vaspin
Visfatin, also pre-B cell enhancing factor (PBEF)
Inflammation
IL-6
IL-1
IL-10
IL-8
Monocyte chemotactic protein-1 (MCP-1)
TNF-alpha
Thrombosis & hypertension
Serum amyloid A (SAA)
CRP
PAI-1, total, active
Proteins of the Renin Angiotensin System
Growth factors
NGF
HGF
Brown fat
Fibroblast growth factor-21
Interleukin-6
Insulin-like growth factor-1

Table 2. *A sampling of adipokines involved in energy balance and metabolism, inflammation, and thrombosis and hypertension.*

Adipokine	Obesity	BBB	Role in Periphery	Role in non-Obese brain	Role in Obese brain
Leptin	↑	Y	Long term regulation food intake (120)	Inhibition of food intake (120)	Leptin resistance (121-127)
			Body weight (120)	Memory formation (112-114, 128)	Deficits in negative feedback loop food intake
			Energy expenditure (120)	Neurogenesis (114, 129)	Impairs memory
				Neuroprotective (130, 131)	Impairs neurogenesis
				Brain structure (132-135)	Impairs neuroprotection Abnormal brain structure
Adiponectin	↓	N	Glucose regulation (136-138)	Neuroprotective (139)	Decreases neuroprotection
			Fatty acid metabolism (136-138) Less inhibition of inflammatory signals (136-138)	Energy expenditure (140)	Decreases energy expenditure
Angiotensinogen	↑	N	Blood pressure regulation (141-146)	Protects BBB (147-149) Regulates arterial Enhances learning & memory(155)	Alters CBF (due to high blood pressure)(146, 150)

Plasminogen activator inhibitor-1	↑	N	Fibrinolysis (158)	Regulates apoptosis (159, 160)	Inhibit BBB fibrinolysis
			Cell migration (158)	Survival of neurites (159)	Increase energy expenditure
			Angiogenesis (158)	Microglial migration (161)	Increase energy intake
				Neuroinflammation (161-166)	
				Brain immune system (161-166)	
				Energy intake (167)	
Interleukin-6	↑	Y	Inflammation (168)	Neuroprotection(169)	Inhibits neurogenesis (170-173)
				NeuroInflammation (170-173)	Decreases synaptic plasticity (170-173)
				Synaptic formation (174)	Disrupts learning & memory processes (170-173)
				Differentiation of neurons and astrocytes (175)	
				Leptin signalling (176, 177)	
Tumor necrosis factor -α	↑	Y	Inflammation (168)	Synaptic transmission (178, 179)	Inhibits neurogenesis (180)
				Synaptic plasticity (181)	Disrupts learning & memory processes (182, 183)
				Neuroinflammation	Increases glutamate excitotoxicity(181)
				Leptin signalling (184)	Damages myelin & oligodendrocytes (185)

Table 3. *Overview of reviewed adipokines, their level in obesity, ability to cross the BBB, and roles in the periphery, non-obese and obese brain. ↑; Plasma levels of adipokine are upregulated in obesity. ↓; Plasma levels of adipokine are downregulated.*

A chronic low inflammation state in the adipose tissue is established in case of excessive WAT accumulation (adiposity), partly mediated via production of pro-inflammatory adipokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (11). Higher plasma IL-6 levels are associated with lower hippocampal gray matter volume (186), and excessive production of TNF-α by WAT could decrease adult neurogenesis by halting cell division of hippocampal progenitor cells, impair long term potentiation facilitation with subsequent memory and learning deficits (77). The release of pro-inflammatory cytokines such as TNF-α and IL-6 might have a pivotal role in the relationship between oxidative stress and endothelial dysfunction (117). Inflammation might have an essential role in the development of insulin resistance, the initiation and progression of atherosclerotic lesions and plaque disruption. Moreover, IL-6 and TNF-α are the main inducers of C-reactive protein (CRP) secretion in the liver. CRP is a marker of low-grade inflammation, and it has been suggested that this protein has a role in the pathogenesis of atherosclerotic lesions in humans (187).

Mouse models

Studies in humans can illustrate an association between adiposity, cognitive function, and related biological variables. However, the exact mechanism behind adiposity related to cognitive impairment cannot be clarified, and therefore translational animal studies are needed. Animal models provide the opportunity to test the efficacy of potential innovative supplementary dietary components in adiposity, anatomical, metabolic, and neurochemical detail. The low-density lipoprotein receptor knock-out Leiden (LDLr-/-) and apolipoprotein E3 Leiden (ApoE3*Leiden) mouse models were chosen in our research because of their adiposity mimicking features when exposed to a diet containing high levels of fat and/or sucrose, such as hyperlipidemia. Secondly, both mouse models were chosen for their vulnerability to develop cardiovascular risk factors often found in obese individuals. ApoE3*Leiden transgenic mice have a rare dominant-negative mutation in the human APOE3 gene and therefore a humanized lipoprotein metabolism. ApoE*3Leiden mice are highly responsive to fat-, sugar- and cholesterol-containing diets, resulting in strongly elevated plasma cholesterol and triglyceride levels with a prominent increase in very low density lipoprotein- and low-density lipoprotein (LDL)-sized lipoprotein fractions (188). LDLr-/- mice lack the functional low-density lipoprotein receptor (LDLr). The LDLr mediates the clearance of cholesterol transported through the bloodstream in low-density lipoproteins by endocytosis (189). LDLr-/- Leiden mice develop obese features on a HFD including hypertension, hyperlipidemia, hypercholesterolemia, increased body weight, and atherosclerosis (190, 191).

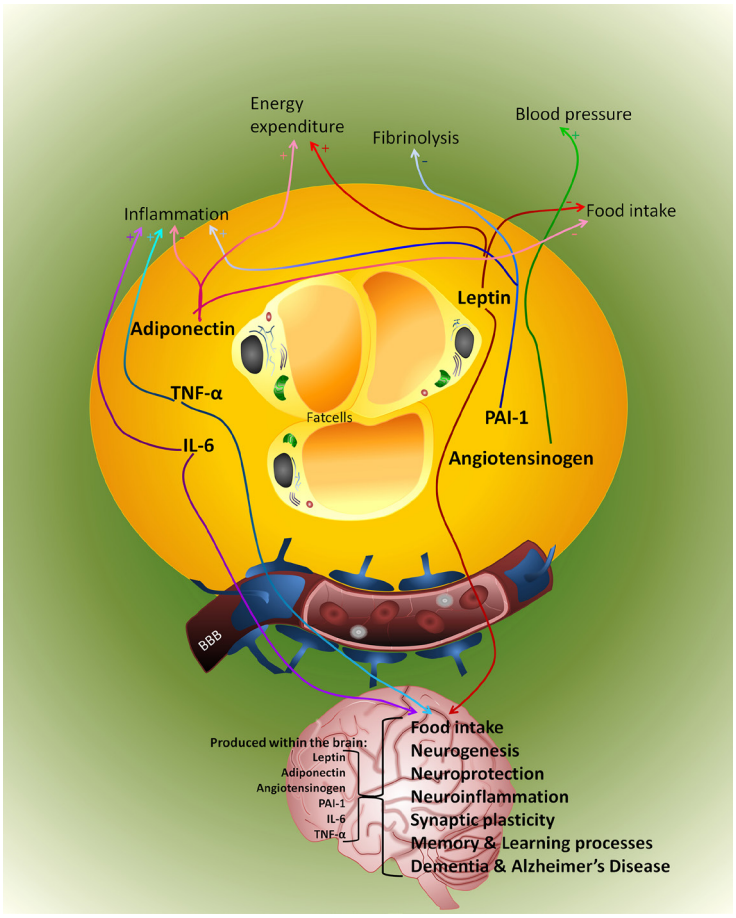

Figure 3. Effects of adipokines in the periphery and brain in adiposity. *Fat cells produce and secrete adipokines like, leptin (red), PAI-1 (blue), angiotensinogen (green), adiponectin (pink), TNF-α (turquoise) and IL-6 (purple). In the periphery, TNF-α and IL-6 stimulate inflammation; these adipokines trigger the liver to produce acute phase proteins. Adiponectin attenuates the inflammatory response by inhibiting the production of TNF-α and IL-6. Furthermore, adiponectin modulates inflammatory responses, energy expenditure (CNS and periphery), food intake (CNS) and a number of metabolic processes, including glucose regulation and fatty acid catabolism. Angiotensinogen increases blood pressure. PAI-1 inhibits fibrinolysis and also stimulates the inflammatory response. Leptin increases energy expenditure and decreases food intake. Only of leptin, TNF-α and Il-6 it is known they are able to cross the BBB and affect, positively or negatively depending on concentration and environment, brain processes such as food intake, synaptic plasticity, learning and memory, and development of dementia. Furthermore, angiotensinogen, PAI-1, IL-6, TNF-α are also produced by neurons, astrocytes and microglia, and several theories exist that leptin and adiponectin might also be produced in the central nervous system. Within the brain these adipokines can be involved in the regulation of food intake, neurogenesis, neuroprotection, neuroinflammation, synaptic plasticity, memory and learning processes and the onset and progression of dementia. Abbreviations: PAI-1: plasminogen activator inhibitor-1; IL-6: interleukin-6; TNF-α:tumor necrosis factor-alpha; CNS: central nervous system; BBB: blood brain barrier; +: increases/ stimulates; -:decreases/inhibit.*

Thesis overview

In this thesis we aim to elucidate the 'adiposity-brain-axis' involving dietary fatty acids (ARA, DHA and butyrate), (cerebro)vasculature, adipokines, and the effect of aging on these processes. In detail the aims of this thesis are:

- Investigate the ability and mechanisms by which specific dietary fatty acids can protect against and counteract HFD induced obesity in mouse models.
- Indicate adiposity associations in aging.
- Examine adiposity in relation to changes in brain structure and dementia in humans.

In **chapter 2** we studied the effect of early life dietary LCPUFAs (ARA and DHA) intake in mild-obese in transgenic ApoE3*Leiden mice. A broad and multidisciplinary experimental approach was used to examine energy metabolism, brain structure, cerebral circulation, and cognition. Therefore, we assessed immunohistochemical studies on fat depots, liver, and brain tissue. Moreover, we tested these mice in behavioral experiments like the open field test and Morris water maze. Neuroimaging was used to determine CBF (using arterial spin labeling (ASL)), functional connectivity (using resting state fMRI), and gray and white matter integrity (using diffusion tensor imaging (DTI)). We hypothesized that the intake of ARA and DHA could attenuate the high fat diet induced effects on metabolism, cerebral blood flow, brain structure and cognition.

The aim of the study in **chapter 3** was to obtain detailed insights in the effects of the short chain fatty acid, butyrate on mid- and late life adiposity in LDLr-/-Leiden mouse model. We hypothesized that the effects of a HFD and butyrate would differ in mid- and late life, according to findings in humans. Metabolism, inflammatory processes, and brain function were extensively examined using blood and microbiome analyses, immunohistochemistry at liver, adipose and brain tissue, the cognitive test the Morris water maze to indicate learning and memory processes, and MRI determining CBF, functional connectivity and brain structure.

In the RUN DMC study (**chapter 4**) we examined adiposity indicators (body mass index (BMI), waist circumference (WC), and blood leptin and total adiponectin levels) in association with brain volumes and components of cerebral Small Vessel Disease (cSVD) in adults age ≥50 years enrolled in the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC study). The RUN DMC study provides a nine year follow-up starting in 2006 of Dutch men and women diagnosed with cerebral small vessel disease.

In the study described in **chapter 5** we analyzed in Gothenburg studies if anthropometric parameters as BMI and waist-hip ratio, and serum adipokines

levels like adiponectin and leptin could predict dementia risk in older Swedish adults over a 10 year period. In addition, we examined whether these associations are different between older men and women. These Gothenburg studies provided us a 10-year prospective population-based study of 70 years and older inhabitants of Gothenburg, Sweden.

Chapter **6 and 7** are dedicated to the general discussion and a summary of all study findings, and we propose suggestions for future studies to gain a better understanding of adiposity and brain function.

2

EARLY INTAKE OF LONG CHAIN POLYUNSATURATED FATTY ACIDS PRESERVE BRAIN STRUCTURE AND FUNCTION IN DIET INDUCED OBESITY

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Abstract

Worldwide, the incidence of obesity is increasing at an alarming rate, and the number of children with obesity is especially worrisome. These developments raise concerns about the physical, psychosocial and cognitive consequences of obesity. It was shown that early dietary intake of arachidonic acid (ARA) and docosahexaenoic acid (DHA) can reduce the detrimental effects of later obesogenic feeding on lipid metabolism and adipogenesis in an animal model of mild obesity.

In the present study, the effects of early dietary ARA and DHA on cognition and brain structure were examined in mildly obesogenic ApoE*3Leiden mouse model. We used cognitive tests and neuroimaging during early and later life. During their early development after weaning (4-13 weeks of age), mice were fed a chow diet or ARA and DHA-diet for eight weeks, and then switched to a high fat and high carbohydrates (HFHC) diet for 12 weeks (14-26 weeks of age).

A HFHC-diet led to increased energy storage in white adipose tissue, increased cholesterol levels, decreased triglycerides levels, increased cerebral blood flow, and decreased functional connectivity between brain regions as well as cerebrovascular and gray matter integrity. ARA and DHA intake reduced the HFHC diet-induced increase in body weight, attenuated plasma triglycerides levels and improved cerebrovasculature, gray matter integrity and functional connectivity in later life.

In conclusion, a HFHC diet causes adverse structural brain and metabolic adaptations, most of which can be averted by dietary ARA and DHA intake early in life supporting metabolic flexibility and cerebral integrity later in life.

Introduction

Each year, obesity or obesity-related conditions lead to the death of 2.8 million people around the world (1). Besides the well-known metabolic complications, obesity is associated with cognitive dysfunction and changes in the central nervous system particularly in the areas of executive function, attention and concentration (77, 88, 94, 192-194). For example, visuospatial organization is affected in overweight children, and in later life these children often suffer from emotional problems (95, 195). It has been shown that obese children also demonstrate an impaired neural activity (196). Remarkably, obese children and adolescents have a decreased gray matter volume of frontal and limbic lobe regions (197). Therefore, novel approaches related to healthy weight development are urgently required.
Animal research has revealed that exposure to high fat diets and high caloric diets increases inflammatory signals within the brain and decreases neurogenesis (198-200). Diets with a high content of fat and carbohydrates (HFHC) are considered to be important contributing factors of overweight and obesity. Promising results in animal and human studies show the potency of long-chain polyunsaturated fatty acids (LCPUFAs) in the prevention and treatment of obesity and overweight (17, 26, 28, 29, 201, 202). The amount and ratio of omega-6 and omega-3 LCPUFAs that are consumed during childhood may determine the adult metabolic profile and risk factors of disease (203). For instance, supplementation with a specific ARA and DHA mixture in early life reduces body weight gain, adiposity and adipose tissue inflammation later in life in mildly obese mice with a lipoprotein profile comparable to humans (202). Moreover, LCPUFAs are essential to support optimal brain and visual system development. Docosahexaenoic acid (DHA) and arachidonic acid (ARA), belonging to the omega-3 and omega-6 LCPUFAs respectively, are the two most abundant LCPUFAs in the brain (reviewed by (204, 205)).

In this study, we therefore studied the effect of early LCPUFA dietary-intake on cognition, brain function and structure in early and later life in ApoE3*Leiden transgenic mice in the context of mild diet-induced obesity. These mice have a humanized lipoprotein metabolism and develop hyperlipidemia and mild obesity on HFHC diets (188, 202, 206). In addition, this mouse model of mild obesity development has not been used yet to evaluate brain development and function earlier. We used a multi-disciplinary experimental approach in order to examine the programming impact of combined dietary ARA and DHA intake in early life on a HFHC diet in later life. Furthermore, we performed *in vivo* and *ex vivo* experiments that cover diet-induced adaptations in energy metabolism, cerebral circulation and cognition. For this purpose, translational cognitive tests and read-outs were applied that are relevant for the human context. The results of this

study are of scientific and societal importance as they further substantiate and elucidate the importance of early programming effects of healthy balanced diets containing LCPUFAs, capable of maintaining metabolic and cognitive flexibility during obesogenic challenges later in life.

Material and Methods

Animals, diets and study design

Male heterozygous ApoE*3Leiden-transgenic mice were housed in individually-ventilated cages (IVC) in conventional animal rooms (relative humidity 50–60%, temperature 21°C, light cycle 7 a.m.–7 p.m. (5 mice per cage)) in the preclinical imaging centre (PRIME) at the central animal laboratory, Radboudumc Nijmegen, the Netherlands. Mice had ad libitum access to acidified tap water and food. These mice originated from the SPF breeding stock at TNO Metabolic Health Research, Leiden, the Netherlands. The mice carry the mutated form of the human apolipoprotein E3 gene, and are considered as a humanized animal model for hyperlipidemia, atherosclerosis and mild obesity. The ApoE*3Leiden mutation is a rare dominant-negative mutation in the human APOE3 gene, and it is characterized by a tandem duplication of codons 120 to 126 and associated with familial dysbetalipoproteinemia. Besides the APOE*3-Leiden gene, this construct consists of the ApoC1 gene and a promoter element that regulates the expression of ApoE and ApoC1 genes (188, 207, 208). ApoE*3Leiden transgenic mice have been generated by introducing a human APOE*3Leiden gene construct into C57Bl/6 mice. ApoE*3Leiden mice are highly responsive to fat-, sugar-, and cholesterol-containing diets resulting in strongly elevated plasma cholesterol and triglyceride levels with a prominent increase in VLDL- and LDL-sized lipoprotein fractions (188). Of note, female mice are prone to develop high plasma cholesterol levels on atherogenic cholesterol-containing Western type diets, while male mice are less susceptible and show plasma cholesterol levels < 8 mM (209). Under the experimental conditions conducted in our current study, the mice do not develop sufficiently high plasma VLDL/LDL cholesterol for development of atherosclerosis.

The total group of 45 ApoE3*Leiden mice was divided into subgroups. During the early life period, from 4 to 13 weeks, 30 mice in the first group received a standardized chow diet (referred to as: '**Chow'**; Sniff R/M diet V1530, Bio Services BV, Uden, The Netherlands). 15 mice were fed a chow diet containing with 0.129% (w/w) ARA and 0.088% (w/w) DHA in their early life (referred to as: '**ARA&DHA'**; DSM Nutritional Products North America, Columbia, MD, USA). In later life, from 14 to 26 weeks, the group on the chow diet was split into a control group (N=15), which continued on the chow diet (referred to as: '**Chow→Chow**'), and

an intervention group (N=15) which switched to a HFHC-diet (referred to as: '**Chow→HFHC**'). The ARA&DHA-group (N=15) also switched to the HFHC-diet later in life (referred to as: '**ARA&DHA→HFHC**'). This HFHC-diet is based on the diets used by Madsen et al. (210). Table 1 provides the composition of the three diets used.

	Chow		ARA&DHA		HFHC	
	% (w/w)	kcal%	% (w/w)	kcal%	% (w/w)	kcal%
Crude protein	19.0	33	19.0	33	16.4	19
Crude fat	3.3	9	3.3	9	28.8	32
Crude fibre	46.1	58	46.1	58	42.6	49
Total		100		100		100
kcal/g		3.8		3.8		4.8
Starch/Sugar	41.2		41.2		36.9	
C14:0	0.01		0.01		0.82	
C16:0	0.47		0.47		6.17	
C16:1	0.01		0.01		0.77	
C18:0	0.08		0.08		5.09	
C18:1	0.62		0.62		8.88	
C18:2	1.80		1.80		5.17	
C18:3	0.23		0.23		0.63	
C20:4	-		0.129		-	
C20-C22	0.01		0.01		0.26	
C22:6	-		0.088		-	

Table 1. Diet composition. *Chow is a conventional diet (Sniff R/M diet V1530, Uden, The Netherlands). In the ARA&DHA diet, the conventional diet is enriched with 0,129% w/w C20:4 (n-6) and 0,088% w/w C22:6 (n-3) fatty acids. The high fat and high carbohydrate (HFHC) diet is a purified diet with a high concentration of carbohydrates and fat, like for instance dextrose, casein and beef tallow (Arie Blok Diets, Woerden, the Netherlands) (50% saturated fat, 46% mono-unsaturated fat and 2 % poly-unsaturated fat). ARA = arachidonic acid; DHA = docosahexaenoic acid; HFHC = high fat & high carbohydrate; (w/w) = weight per weight; kcal% = percent of kilo calorie.*

Blood samples were taken after 5 hours of fasting (8 a.m.–1 p.m.) at 4, 10, 12, 20 and 26 weeks of age. These blood samples were tested for plasma triglycerides, cholesterol, insulin and leptin levels. Individual body weight and food intake (at cage level) were monitored over time. To investigate possible changes in cognition and brain structure induced by diet in early life, the mice, aged 12-13 weeks, were assessed in several cognitive tests and MRI experiments. The following experiments were performed: open field, rotarod, object recognition test (ORT), resting state functional MRI (rsfMRI), arterial spin labeling (ASL), and diffusion tensor imaging (DTI). In early life, all mice were assessed in cognitive tests, and 20 chow and 10 ARA&DHA fed mice were examined in MRI experiments. Thereafter, the mice switched to a HFHC diet. In later life these mice, aged 26 weeks, were assessed in a second run of the same cognitive tests and MRI experiments, but only the ORT was replaced for the long term memory test, Morris water maze (MWM). Ten mice per diet group were assessed for MRI experiments, and fifteen mice per diet group were examined during the cognitive tests. At 26 weeks of age, all mice were anesthetized and sacrificed by transcardial perfusion with 0.1M phosphate-buffered saline (PBS), and thereafter organs like brain, liver and adipose tissue were harvested. An overview of the study design is provided in figure 1.

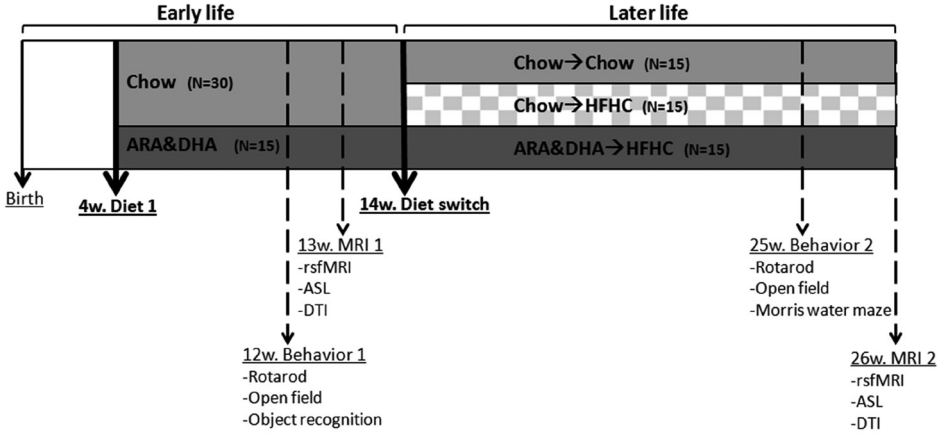

Figure 1. Study design. *In early life, ApoE3*Leiden mice received either a chow diet or an ARA&DHA-diet. The first run of cognitive tests was assessed in all mice aged 12 weeks, i.e. open field, rotarod and object recognition test. The following week, these mice were assessed in MRI-experiments (Chow N=20 and ARA&DHA N=10). At 14 weeks of age, the mice switched from diet. All 25 week-old mice were assessed for the second run of cognitive testing, and MRI-experiments (N=10 per diet group). All mice aged 26 weeks were anesthetized and sacrificed by transcardial perfusion. HFHC: high fat and high carbohydrate diet; rsfMRI: resting state functional magnetic resonance imaging; ASL: arterial spin labelling and DTI: diffusion tensor imaging.*

Plasma analyses
Total plasma cholesterol and triglyceride levels were measured enzymatically using kits number 11489437 and 11488872 (Roche Diagnostics, Almere, The Netherlands) following a standardized protocol of *Wielinga et al.* (202).

Open field
The open field test (OF) was performed to analyze locomotion and exploration. We followed a protocol previously described by *Janssen et al.* (211-213).

Rotarod
To test sensory-motor integration, all mice were placed on a rotating drum to assess in the rotarod test. The rotarod was performed and analyzed following a standardized protocol described by *Janssen et al.* (213).

Object recognition test
ApoE3*Leiden male mice were assessed in the object recognition test (ORT) to examine short term memory. This method was based on literature of *Brooks et al., Bevins and Besheer* (56, 214). In short, subsequent to habituation, two similar objects were placed in the box and a mouse was allowed to explore the objects after a 30, 60 and 120 minutes delay, respectively. All short term memory trials were recorded, and afterwards blind and randomized scored using Ethovision (EthoVision XT 8.5, Noldus Information Technology, Wageningen, The Netherlands). A mouse was considered as an outlier, if it had no interest in the objects during the two memory trials, or if it sat at the objects. Preference for the novel object was expressed in discrimination ratio, which can be defined as: the time spent exploring the novel object minus the familiar object divided by the total object explorer-time.

Morris water maze
Acquisition and probe
The Morris water maze (MWM) was assessed to examine long term memory and spatial learning abilities of the ApoE3*Leiden mice aged 25 weeks. Mice had to seek a submerged platform located in the North-East (NE) quadrant in a circular pool (104 cm diameter) filled with water (21–22°C; made opaque by the addition of milk powder), as previously described by *Janssen et al.* (213).

Search strategies
During the MWM acquisition phase, mice use different search strategies in context of spatial learning. During subsequent trials mice proceed from random towards hippocampus-dependent, and more direct and precise search strategies (215, 216). These swimming pathways were analyzed, and classified into seven search strategies

in order to indicate hippocampus-dependent and -independent search strategies (Ethovision XT 8.5, Noldus Information Technology, Wageningen, Netherlands).

Immunohistochemistry

The left hemisphere of the brain was postfixed in 4% paraformaldehyde for 24 hours for immunohistochemical purposes. Afterwards the hemispheres were stored in 0.1M phosphate buffered saline (PBS) with 0.01% sodium azide (NaN_3). Coronal brain sections were sectioned using a sliding microtome (Microm HM 440, Walldorf, Germany) equipped with an object table for freeze sectioning at -60°C gaining 8 series of 30-µm thick sections. The right hemispheres were divided into 4 sections and immediately snap frozen and stored in a -80˚C freezer. Adipose tissue was also divided and stored for either immunohistochemical or biochemical purposes.

Adipose tissue analysis

Adipose tissues were fixed in formalin, embedded in paraffin and sliced in 5 µm sections. The slices were stained with hematoxylin. Briefly, were photographed with a Zeiss Imager A2 microscope (Zeiss, Germany) at 20x magnification with Ludl 3-axis controlled stepping motor system with a colour digital camera. Quantification was performed with ImageJ software (JAVA based public domain, National institutes of health, Maryland, USA) which calculated the size of the adipocytes (μm^2) for three sections of each fat depots, and around the 30-40 adipocytes per section.

Glucose transporter-1 fluorescent immunohistochemistry

To measure the amount of glucose transporters-1 (GLUT-1) in the blood vessel walls in the brain indication for blood vessel integrity, an intensity staining was used. We used a free-floating fluorescent staining of GLUT-1 on hippocampal sections of 30 µm. Sections reserved for the fluorescence GLUT-1 staining were pre-incubated for half an hour in PBS-BT followed by the primary incubation overnight with rabbit anti-GLUT-1 (1:5000; Millipore, Billerica, Massachusetts, USA) at room temperature. After the primary incubation, the sections were rinsed and then incubated with the second antibody, donkey anti-rabit-Alexa-445 conjugated (1:100 in PBS-BT; Jackson Immuno Research, Suffolk, UK), for three hours in the dark. Subsequently, sections were rinsed, mounted on gelatin coated slides and air-dried in the dark for two hours. After embedding the sections in Fluorsave the sections were stored at 7 °C. Pictures were taken at a 5x magnification with an Axio Imager A2 (Zeiss Germany) and the sections were stored for a longer period at -20 °C. Sections were photographed using a shutter speed of 500 ms at 470 nm to excite the fluorescence. ImageJ was used for quantification of amount of positive glucose

transporters in blood vessels in the cortex, thalamus and hippocampus (bregma -1.7 and -2.46). In addition, the surface and intensity were measured.

Quantitative real-time polymerase chain reaction

The snap-frozen midbrains were used for the analysis of mRNA levels using quantitative real-time polymerase chain reaction (qRT-PCR) of: glucose transporters-1 (GLUT-1, (217)), preproinsulin (218), insulin receptor (219), leptin receptor A (220) and B (221), vascular endothelial growth factor (VEGF, (222)), occludin (223). Forward and reverse primer sequences are represented in table 2. A standardized protocol was used for these analyses as previously described by *Janssen et al.* (213). Per primer a negative control consisting of water instead of cDNA was added and per primer one plate was used consisting of both the glyceraldehyd 3-phosphate dehydrogenase (GAPDH) and the primer of interest. GAPDH is a reference gene serving as a control for general cell activity. The quality of the samples was evaluated using the melt curve and amplification plot. The StepOne Software version 2.2.2 was used to determine the threshold cycle (CT) values. To analyze possible differences between dietgroups CT values were normalized by GAPDH and transformed to the comparative CT value via the 2-ΔCT method (224).

Primer	Sequence	
GAPDH	FW	5'-AGGTCGGTGTGAACGGATTTG-3'
	REV	5'-TGTAGACCATGTAGTTGAGGTCA-3'
GLUT-1	FW	5'-TCAACACGGCCTTCACTG-3'
	REV	5'-CACGATGCTCAGATAGGACATC-3'
Occludin	FW	5'-AGACTACACGACAGGTGGGG-3'
	REV	5'-CTGCAGACCTGCATCAAAAT-3'
VEGF-A	FW	5'-GGAGATCCTTCGAGGAGCACTT-3'
	REV	5'-GGCGATTTAGCAGCAGATATAAGAA-3'
ObRA	FW	5'-ATGGGCTGTGATCGGAACTG-3'
	REV	5'-GTCTTCCCAATAAGCATGTCTCC-3'
ObRB	FW	5'-GTTCCAAACCCCAAGAATTG-3'
	REV	5'-TGCTCAAATGTTTCAGGCTTT-3'
Preproinsulin	FW	5'-ATAAAGCTGGTGGGCATCCA-3'
	REV	5'-GCACCAACAGGGCCATGT-3'
InsulinR	FW	5'-ATGGGCTTCGGGAGAGGAT-3'
	REV	5'-GGATGTCCATACCAGGGCAC-3'

Table 2. Forward and reverse sequences of the primers. *GAPDH: glyceralehyde-3-phosphate dehydrogenase; GLUT-1: glucose transporters-1; Occludin: tight junction protein; VEGF: vascular endothelial growth factor; ObRA: leptin receptor A; ObRB: leptin receptor B and InsulinR: insulin receptor; FW: forward; REV: reverse.*

MRI-experiments

MRI measurements were performed with a 11.7 T Biospec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) equipped with an actively shielded gradient set of 600 mT/m, and operated by a Paravision 5.1 software platform. We used a circular polarized volume resonator for signal transmission and an actively decoupled mouse brain quadrature surface coil for signal reception (Bruker BioSpin). During the experiment, isoflurane (Nicholas Primal (I) limited, London, United Kingdom) was used for anaesthesia (3.5% for induction for 2 minutes and approximately 1.8% for maintenance in a mixture of oxygen and nitrous oxide (1:2)). Respiration of the animal was monitored using a pneumatic cushion respiratory monitoring system (Small Animal Instruments Inc., NY, USA). Mice were placed in a stereotactic device to immobilize the head. Body temperature was measured using a rectal thermometer and maintained at 37 °C using a heated air flow device. First, gradient echo images were acquired using previously described image parameters (225). Resting state fMRI (rsfMRI) acquisition and analysis were performed to assess functional connectivity within specific brain regions following a protocol of *Zerbi et al.*(226). Arterial spin labeling (ASL) was assessed to indicate cerebral blood flow (CBF) levels using an established ASL method with flow-sensitive alternating inversion recovery (FAIR) technique (227-229). ALS method was performed as previously described by (225, 226). Measurements were assessed in two standardized gas concentrations, first: 2 O_2 : 1 N_2O, and the second, vasoconstriction inducing concentration, 3 O_2 : 0 N_2O. CBF values from both conditions were used to examine the ability of the cerebrovasculature to adapt from a normal condition into a vasoconstrictive condition, which is referred to as vasoactivity. To calculate vasoactivity, the normal CBF values were abstracted from the vasoconstriction CBF values, and this was then divided by the sum of the normal and vasoconstrictive CBF values. To calculate regional CBF we used the same protocol as previously described (225). Finally, diffusion tensor imaging (DTI) was performed to determine gray and white matter integrity following a modified protocol of *Zerbi et al.* (230). All imaging parameters are represented in table 3.

Statistical analysis

A random and double-blind selection procedure was maintained throughout the experiment. The results are expressed as means ± standard error of mean (SEM). Means were analyzed using repeated measures, univariate or multivariate analysis of Analysis of variance (ANOVAs) with Bonferroni correction for multiple testing in a statistical program, SPSS20 (IBM SPSS Statistics 20, IBM Corporation, Armonk, New York, USA). Diet effects were referred to as significant if the P-value was lower than 0.050, and a trend was implied if the P-value was below 0.080 and above 0.050.

Statistical analysis were only performed on the basis of tests for normality (231) and homogeneity of variance (232). All Data are presented in Mean ± SEM.

	Imaging sequences			
	Anatomical T2*w	**ASL**	**DTI**	**rsfMRI**
Imaging method	GE	FAIR-ASL	4-shot spin-echo PI	Spin-echo EPI
Echo time (ms)	5	11.8	20	16.9
Repetition time (s)	0.63	13.75	7.55	1.8
Image matrix (pixel x pixel)	512x512	128x128	128x128	96x96
Field-of-view (mm)	40x40	30x30	20x20	25x25
Spatial resolution (μm/pixel)	78x78x340	234x234x 1000	156x156x500	260x260x500
Number of slices	20x3	1	22	9
Total acquisition time (min)	8	12	18	18

Table 3. List of parameters used in MRI experiments

Results

Metabolic parameters

Body weight and food intake

At the start of the experiment, when the mice were four weeks of age, the averaged body weight was 14.9 ± 0.36g. Up to 13 weeks, body weight measurements revealed that ApoE3*Leiden mice on ARA&DHA-diet had a significantly (P=0.028, F(1,43)=5,16) lower body weight compared to the mice on chow-diet (Fig. 2A). Food intake was measured per cage (N=3 per diet group), and in early life, from 4 to 13 weeks of age, these mice consumed about the same amount of chow or ARA&DHA-diet (Chow: 4.1±0.052 g diet/mouse/day; ARA&DHA: 3.84±0.052 g diet/mouse/day).

After the diet switch at 14 weeks, mice fed the ARA&DHA→HFHC diets tended to maintain a lower body weight when compared to Chow→HFHC fed mice (P=0.080, F(1,28)=3.14) (Fig. 2). The switch to a HFHC-diet caused a significantly reduced food intake in these mice in later life, possibly because rodents often maintain an iso-caloric eating pattern (Chow→Chow: 3.13±0.33; Chow→HFHC: 1.54±0.33 ; ARA&DHA→HFHC: 1.50±0.22; P=0.007, F(1,4)=12.42).

Plasma analysis

Moreover, as reported previously by *Wielinga et al*., the ARA&DHA treated mice showed a decreased plasma cholesterol level compared to chow fed mice (Chow: 3.01±0.08 mM; ARA&DHA: 2.71±0.07 mM; P=0.02, F(1,43)= 5.92). Also plasma triglyceride concentrations were significantly decreased in the ARA&DHA fed mice (Chow: 2.32±0.09 mmol/L; ARA&DHA: 2.01±0.01 mmol/L; P=0.03, F(1,43)= 5.20). Plasma insulin levels were below 0.5 ug/mL in early life and comparable in both groups (Insulin: Chow: 0.019±0.00 ug/mL; ARA&DHA: 0.031±0.00 ug/mL), whereas plasma leptin concentrations were below detectable for both diets.

At 26 weeks of life, plasma cholesterol levels remained stable in the Chow→Chow diet group up to 20 weeks, whereas the plasma cholesterol concentrations increased in the Chow→HFHC and ARA&DHA→HFHC diet group (Chow→Chow: 2.92±0.08 mM; Chow→HFHC: 6.23±0.01 mM; ARA&DHA→HFHC: 6.45±0.12 mM; P=0.00 F(1,28)=476,25). Plasma triglycerides concentrations decreased in all HFHC fed mice, compared to chow fed mice (Chow→Chow: 2.19±0.30 mmol/L; Chow→HFHC: 1.07±0.02 mmol/L; ARA&DHA→HFHC: 1.12±0.02 mmol/L; P=0.00, F(1,28)=184,07). Moreover, plasma insulin levels remained low for all diet groups (Chow/Chow: 0.63±0.06 ug/mL; Chow→HFHC: 0.46±0.12 ug/mL; ARA&DHA→HFHC: 0.50±0.11 ug/mL). Leptin plasma levels increased in the mice fed a HFHC diet (Chow→Chow: 1.00±0.30 ng/mL; Chow→HFHC: 2.08±0.58 ng/mL; ARA&DHA→HFHC: 3.14±0.46 ng/mL; P=0.10, F(1,28)=2,87).

Liver, white adipose tissue and brain tissue analysis

At 26 weeks all mice were sacrificed, and organs harvested and weighed. Liver weight was significantly decreased in the Chow→HFHC-diet group compared to the Chow→Chow-group (P<0.001, F(1,28)=32.63; Fig. 2B), whereas epididymal and inguinal fat weights were significantly increased in the Chow→HFHC-group compared to the Chow→Chow-group (P=0.001, F(1,28)=12.97; P=0.010, F(1,28)=7.55; Fig. 2B).

In order to determine whether the storage of lipids was indeed increased in adipocytes due to HFHC feeding, we measured the size of adipocytes in omental, epididymal and inguinal fat depots. In later life, mice fed a HFHC diet had an increased adipocyte size in all three analyzed fat depots, versus chow fed mice (Omental: P=0.01, F(1,28)=6,84; Epididymal: P<0.001, F(1,28)=14,88; Inguinal: P=0.03, F(1,28)=5,41; Fig. 2C). Only in the inguinal fat depot, the adipocyte size of mice fed ARA&DHA was reduced, when compared to HFHC fed mice (P=0.05, F(1,28)=4,27; Fig. 2C).

In order to reveal changes in insulin and leptin metabolism in the brain, we performed biochemical analysis of the mice brain tissue. No significant diet effects were found in mRNA levels of the leptin receptors A and B nor in mRNA levels of preproinsulin and the insulin receptor (data not shown).

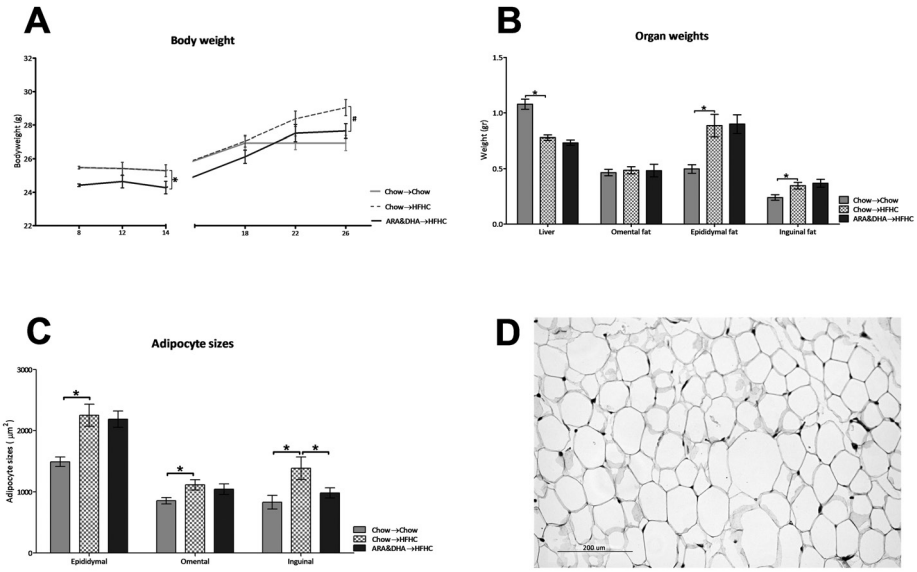

Figure 2. Metabolic parameters. *A) Body weight. In early life, the ARA&DHA-group (N=15) showed a reduced body weight when compared to the Chow-group (N=30). In later life, mice on the ARA&DHAHFHC diets had a decreased body weight compared to the Chow→HFHC-group (N=15 per diet group). B) Organ weights at 26 weeks of life. Liver weight was reduced in the Chow→HFHC-dietgroup. Epididymal and inguinal fat weight was increased in the Chow→HFHC-mice (N=15 per diet group). C) Adipocyte sizes in omental, epididymal and inguinal fat depots. HFHC feeding induced an increase in adipocyte size in omental and epididymal fat depots. Early life ARA&DHA dietary intake reduced the adipocyte size within inguinal fat depots (N=15 per diet group). D) Representative image of histologically (H&E) stained fat tissue. *= P≤0.05; #=0.08.*

Cerebrovascular parameters

CBF analysis

We performed ASL-measurements to indicate CBF levels in the brains of ApoE3*Leiden mice (Fig. 3). In early life, mice fed the ARA&DHA-diet demonstrated an increased vasoactivity within the cortex, compared to the mice fed a chow-diet (P=0.034, F(1,25)=5,04) (Fig. 3C). These results illustrate an increased ability of the cerebrovasculature to react on vasoconstrictive stimuli.

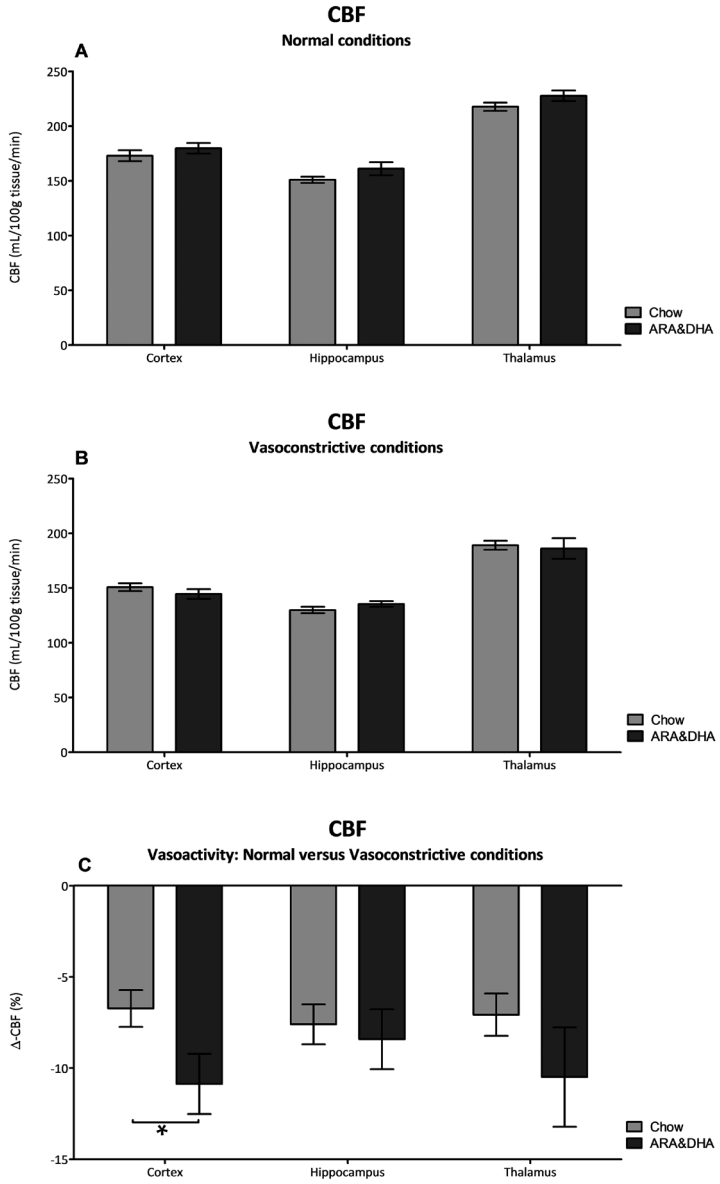

Figure 3. CBF-levels and vasoactivity in early life. *A) CBF-levels. B) CBF-levels under vasoconstrictive conditions. C) Vasoactivity. It represents the ability of the cerebrovasculature to react on vasoconstrictive stimuli. Mice fed ARA&DHA had an increased vasoactivity in the cortex. Chow: N=18; ARA&DHA: N=9; *= P≤0.05.*

In 26 week-old mice the CBF level was significantly increased in the Chow→HFHC-diet group within the cortex (P=0.044,(F(1,14)=4,87) and thalamus (P=0.017, F(1,14)=7,33) compared to the Chow→Chow-diet group (Fig. 4A). In addition, a trend for increased CBF-levels of mice on Chow→HFHC diets compared to mice on ARA&DHA→HFHC-diets was found in the thalamus (P=0.074, F(1,17)=3,62; Fig. 4). In vasoconstrictive conditions, the Chow→HFHC-group revealed again a significant increase in CBF levels in the cortex (P=0.009, F(1,14)=7,33), hippocampus (P=0.054, F(1,14)=4,43) and thalamus versus Chow→Chow (P=0.014, F(1,14)=7,86) (Fig. 4B). No significant diet effects were revealed on the vasoactivity at 26 weeks.

Immunohistochemistry GLUT-1

GLUT-1 is a transporter protein for glucose located in the blood vessel wall and can be used to elucidate vascular integrity in the brain. GLUT-1 fluorescent immunohistochemistry demonstrated significant effects on the number of GLUT-1 positive blood vessels per μm^2 (Fig. 4C). The ARA&DHA→HFHC fed mice showed an increased number of blood vessels compared to the Chow→HFHC fed mice in the cortex (P=0.025, F(1,28)=5.64), hippocampus (P=0.019, F(1,28)=6.18) and thalamus (P=0.017, F(1,28)=6.45) (Fig. 4C). Chow→HFHC fed mice also had less GLUT-1 positive blood vessels compared to the Chow→Chow fed mice within the hippocampus (P=0.078, F(1,28)=3.35) and thalamus (P=0.072, F(1,28)=3.50) (Fig. 4C). The amount of GLUT-1 protein within the cortex, hippocampus and thalamus was analyzed by intensity measurements, and mRNA levels of GLUT-1 were analyzed using qRT-PCRs. In both measurements no significant diet effects were found, indicating that the GLUT-1 protein and mRNA expression within the blood vessel wall was unchanged by diet while number of blood vessels was changed by diet. In addition, we performed biochemical experiments (qRT-PCRs) to analyze the blood brain barrier and vasculogenesis in the brain. Occludin and vascular endothelial growth factor A (VEGF-A) were examined, and yet no significant diet effects were found (data not shown).

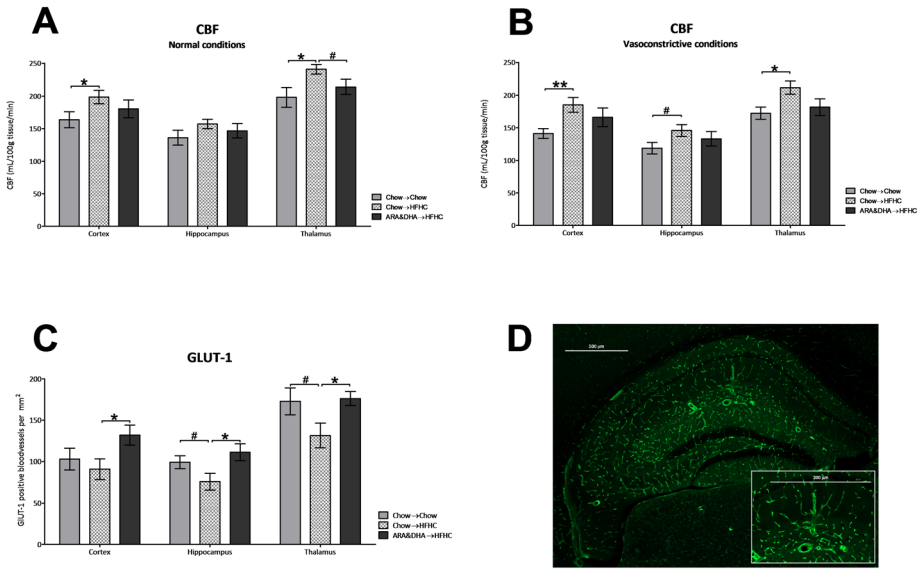

Figure 4. Cerebrovascular parameters in later life.
A) CBF-levels. Chow→HFHC-diet group had an increased CBF-level within the cortex and thalamus. ARA&DHA→HFHC fed mice demonstrated decreased CBF levels in the thalamus. (Chow→Chow: N=7; Chow→HFHC: N=9; ARA&DHA→HFHC: N=10) B) CBF-levels under vasoconstrictive conditions. Chow→HFHC fed mice had increased CBF levels in the cortex, hippocampus and thalamus. (Chow→Chow: N=7; Chow→HFHC: N=9; ARA&DHA→HFHC: N=10) C) Quantification GLUT-1 immunohistochemistry in the cortex, thalamus and hippocampus. GLUT-1 is a transporter protein located in the blood vessel wall and is used as a marker for cerebrovascular integrity. Chow→HFHC-group showed a significant decreased number of GLUT-1 positive blood vessels in the cortex, hippocampus and thalamus. (N=15 per diet group) D) A representative immunofluorescent image of a GLUT-1 stained hippocampal section.= P≤0.05; #≤0.08.*

Cognitive parameters

Behavioral experiments

In early life, we examined locomotion, exploration, short term memory and sensory motor integration in the open field, ORT and rotarod tests. In none of these parameters a significant diet effect was revealed between mice fed the chow or ARA&DHA-diet.

In later life, mice were tested in the open field and rotarod, and again no significant diet effects were found in locomotion, exploration and sensory motor integration. A long term memory test, the MWM, was performed in mice aged 25 weeks. During acquisition, all mice learned and found the platform location. Analyses of the search strategies revealed a significant diet effect (P=0.020, F(1,28)=6.30); the ARA&DHA→HFHC fed mice used an increased level of spatial-hippocampus

dependent search strategies compared to the Chow→HFHC fed mice (Fig. 5A). In the probe trial, ARA&DHA→HFHC fed mice had a decreased swimming distance (P=0.042, F(1,28)=4.55) and velocity (P=0.038, F(1,28)=4.73) compared to Chow→HFHC fed mice.

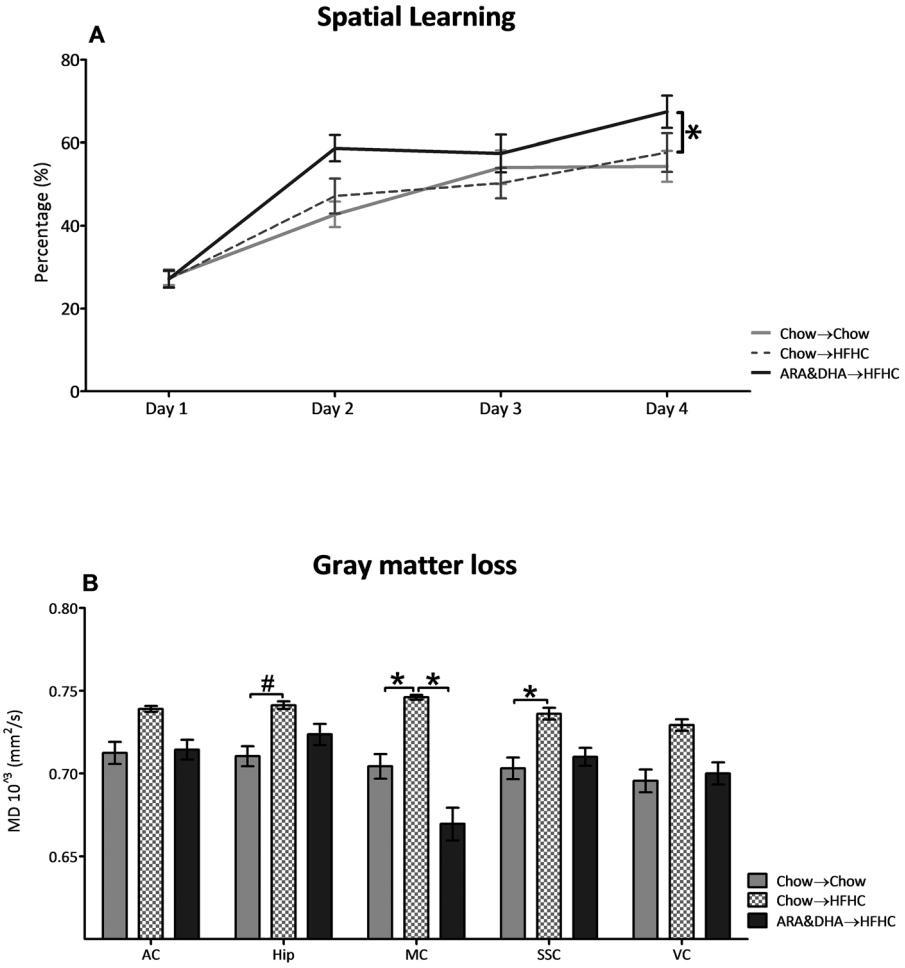

Figure 5. MWM learning and memory, and DTI gray matter integrity in later life.
*A) Classification of the search strategies in the MWM. ARA&DHA→HFHC-group had an increased hippocampal dependent spatial learning-ability. Y-axis displays percentage of used hippocampal dependent search strategy (↑ percentages= more use of a hippocampal dependent search strategy, N=15 per diet group) B) Mean diffusivity (MD) levels of mice aged 26 weeks. Chow→HFHC-group had increased MD levels in the hippocampus, motor cortex and somatosensory cortex, which indicates a decreased gray matter integrity. (Chow→Chow: N=6; Chow→HFHC: N=8; ARA&DHA→HFHC: N=9) *= P≤0.05; #≤0.08 AC: Auditory cortex; Hip: Hippocampus; MC: Motorcortex; SSC: Somatosensory cortex; VC: Visual cortex.*

DTI and rsfMRI

DTI-experiments were assessed to determine white and gray matter integrity via fractional anisotropy (FA) and mean diffusivity (MD) levels; no significant diet effects were revealed in FA or MD levels in early life at 13 weeks. However, functional connectivity, assessed by rsfMRI, was increased in mice fed a ARA&DHA-diet in early life. These diet effects were mainly found in functional connections between the following brain regions: auditory, visual, motor and somatosensory cortex (Fig. 6A-B).

In the second set of MRI experiments at 26 weeks of life, DTI parameters, FA and MD levels, were again used to analyze white and gray matter integrity. No significant effects were found in FA-levels, while MD-levels of Chow→HFHC fed mice were affected compared to Chow→Chow fed mice. More specifically, the Chow→HFHC-diet group showed an increased MD-level within the hippocampus ($P= 0.059$, $F(1,12)=4.35$), motor cortex ($P= 0.026$, $F(1,12)=4.35$) and somatosensory cortex ($P= 0.049$, $F(1,12)=4.82$) versus only Chow fed mice (Fig. 5B). In the motorcortex, the MD-levels of the Chow→HFHC fed mice were also significantly increased ($P= 0.024$,$F(1,15)=5.80$) when compared to ARA&DHA→HFHC (Fig. 5B). Together, these results may indicate neuronal loss and decreased gray matter integrity in the Chow→HFHC fed mice in these areas (Fig. 5B). rsfMRI analysis revealed a decreased functional connectivity in Chow→HFHC relative to Chow→Chow. These significant diet effects were found in the connectivity between the ventral hippocampus, motor and somatosensory cortex (Fig.6C-D). Compared to the Chow→HFHC fed mice, the ARA&DHA→HFHC fed mice showed an increased functional connectivity between the ventral hippocampus, motor and somatosensory cortex (Fig. 6E-F).

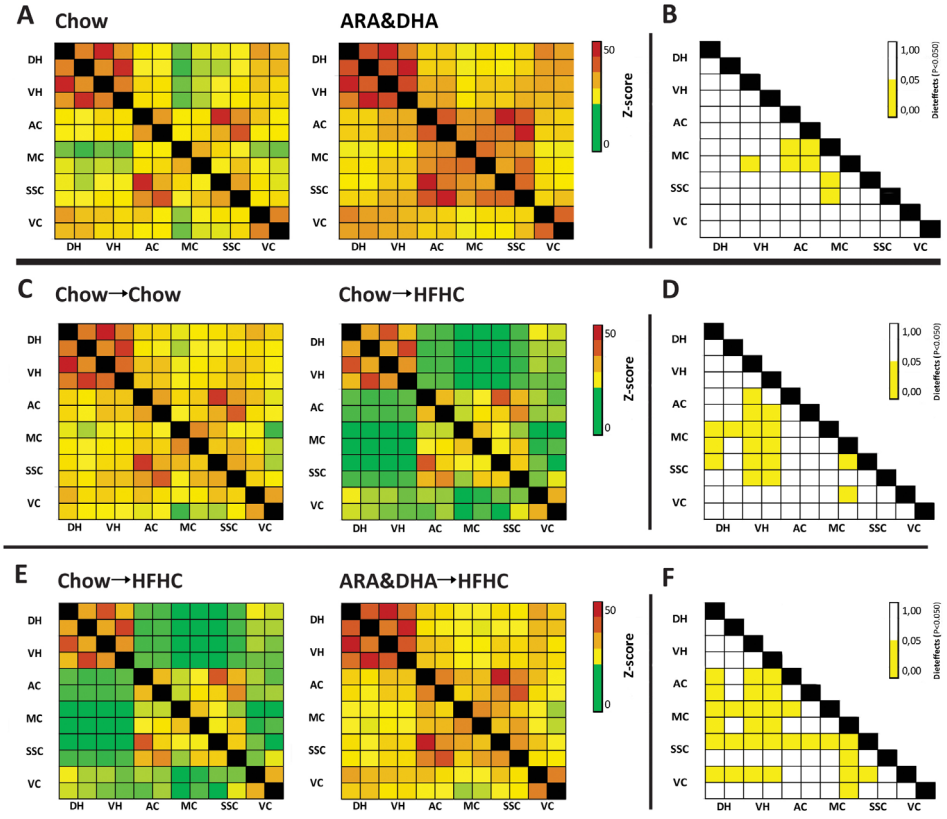

Figure 6. Early and later life connectivity between brain regions using rsfMRI.
A) Total correlation matrixes of the Chow and ARA&DHA diet group at 13 weeks of life. C-E) In later life at 26 weeks, the total ROI matrixes of the Chow→Chow, Chow→HFHC or ARA&DHA→HFHC fed mice. The selected brain regions, dorsal hippocampus (DH), ventral hippocampus (VH), auditory cortex (AC),motor cortex (MC), somatosensory cortex (SSC) and visual cortex (VC), are subdivided in left hemisphere region (first row) and right hemisphere region (second row). In general the DH and VH are strongly bilaterally connected, and AC, MC and SSC are highly interconnected. A higher Z-score (red) indicates a stronger functional connectivity. B-D-F) Statistical analyses of functional connectivity to reveal a significant diet effect are also represented as a matrix. Yellow: P<0.050 . A) In early life, ARA&DHA fed mice had an increased functional connectivity between the motor, visual and somatosensory cortex. B) Statistical matrix of Chow vs ARA&DHA in early life. (yellow= P<0.050) C) The Chow→HFHC-group has a decreased functional connectivity between the ventral hippocampus, motor cortex and somatosensory cortex. D) Statistical matrix of Chow→Chow vs Chow→HFHC in later life. (yellow= P<0.050) E) Functional connectivity was increased in ARA&DHA→HFHC fed mice compared to Chow→HFHC fed mice between the ventral hippocampus, auditory, motor and somatosensory cortex. F) Statistical matrix of Chow→HFHC vs ARA&DHA→HFHC in later life. (yellow= P<0.050) Chow: N=20; ARA&DHA: N=10; Chow→Chow: N=7; Chow→HFHC: N=7; ARA&DHA→HFHC: N=7.

Discussion

In the present study, we are the first to show that a mild obesogenic HFHC diet influences brain structure and function by increasing CBF, decreasing gray matter integrity and functional connectivity. Secondly, brain structure and function can be preserved by an early life ARA&DHA dietary intake. Moreover, early life programming is important in the etiology of mild obesity.

Early life

Fetal and neonatal time periods are critical time windows in development. Nutrition in these critical periods can have long-term phenotypic consequences through permanent structural changes in organs, which is associated with the development of long term obesity (233-237). Furthermore, the amount and ratio of omega-6 and omega-3 LCPUFAs that are consumed during childhood may determine the adult metabolic profile and risk factors of disease (203). Failure to provide LCPUFAs in these critical developmental periods results in alterations in visual function, spatial learning and memory, nerve growth factor levels and dopamine production, as well as decreased glucose utilization in the brain (238-245). These effects could be related to, for instance, the amount of DHA incorporated in the neuronal membranes, because DHA can change the physicochemical properties of membranes, and deficiency of DHA can impair G-protein receptor-mediated signalling in neural membranes (238, 242, 244, 245). Furthermore, *Gomez-Pinilla et al.* showed that dietary DHA intake elevated the amount of leptin receptor b in the hypothalamus and hippocampus, but did not affect ghrelin receptors indicating that DHA specifically influences leptin signalling in the hypothalamic appetite system (246). Of note, the development of the hypothalamic appetite regulatory network occurs predominantly after birth (247). It is well established that ghrelin affects the orexigenic Neuropeptide Y (NPY) and Agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus resulting in an increased appetite (248, 249). In contrast, leptin inhibits the NPY and AgRP neurons and activates the anorexigenic neurons of the melanocortin (α-MSH)/cocaine and amphetamine-regulated transcript (CART) pathways in the arcuate nucleus, which results in decreased food intake and increased energy expenditure (248). ARA metabolites can modulate hypothalamic corticotropin-releasing hormone secretion, and induce a long-term activity-dependent enhancement of synaptic transmission in the hippocampus (16, 250). Thus, the hypothalamus works in concert with the hippocampus to regulate energy homeostasis, food intake and cognitive function (251-253). Treatment with ARA and DHA in early life may affect one or several of these key regulatory processes of metabolic homeostasis, and may involve effects in hypothalamus and hippocampus. The observed effects suggests that LCPUFAs like ARA and DHA exhibit promising effects in the prevention and treatment of obesity

and the metabolic profile, possibly via effects on the hypothalamic appetite system, already in early life.

Metabolism

In early life, ApoE3*Leiden mice fed LCPUFAs gained significantly less weight, and plasma cholesterol and triglycerides levels were decreased. These mice still had a lower body weight after 3 months of HFHC diet feeding, despite increased plasma cholesterol and leptin levels. In later life, we observed in all HFHC treated mice a decreased liver weight, while inguinal and epididymal fat weight increased. These results indicate a changed lipid storage in the HFHC-fed mice, with lipids possibly being stored into the epididymal and inguinal fat tissue instead of in the liver (254). Some studies reported an increased liver weight in mice after high fat and /or high carbohydrate feeding (210, 255), and most of them described an increased fat accumulation in the hepatic cells in the context of NAFLD (256-258). Although, *West et al.* found that an obesity inducing diet did not significantly increase the liver weight in nine inbred mice strains (259). In contrast, in our current study we found a decreased liver weight in later life. This effect appeared not to be related to ARA&DHA treatment. The observed reduction in liver weight upon switching from chow diet to a fat-containing obesogenic diet may be related to an effect on *de novo* lipogenesis in combination with a preferred storage of fat in white adipose tissue during obesogenic diet feeding. Under chow conditions energy comes mainly from carbohydrates and only in little amounts from dietary fat. Therefore, *de novo* lipogenesis is active to produce the fatty acids that are needed for organ function (e.g. heart). When animals are switched to obesogenic diets containing more dietary fat, *de novo* lipogenesis is reduced in the liver, and fat storage processes are activated in adipose tissue (206, 260). In parallel, under high fat diet conditions, fat storage in white adipose tissue is activated and, associated with this, liver fat and mass decline as storage pools in adipose tissue are filled. Indeed, we observe an increase in epidydimal WAT tissue after the switch from chow to high fat diets. Of note, under more extreme conditions these initially physiological responses may develop into more pathophysiological responses in liver and adipose tissue, ultimately leading to adipose tissue hypertrophy and inflammation and associated hepatic steatosis with increased liver weights as shown recently in longitudinal studies of diet-induced obesity, adipose tissue inflammation and NAFLD (260, 261). ARA&DHA fed mice displayed reduced adipocyte size within the inguinal fat depot. In agreement, *Wielinga et al.* reported that inguinal adipocyte cells were smaller in ApoE*3Leiden mice with early life ARA&DHA intake after eight weeks of HFHC feeding (202). It is hypothesized that a reduced adipocyte size due to early ARA&DHA supplementation could be a result of variations in adipocyte differentiation, as expression profiling revealed significant differences in expression of hundreds of genes between different adipose depots in rodents (262). These

findings confirm that ARA&DHA dietary intake in early life strengthens the ability to cope with a mild obesogenic diet later in life.

Cerebrovasculature

LCPUFA intake improved the vasoactivity in the brains of ApoE3*Leiden mice in early life. In monkeys, a PET study revealed that DHA-supplementation for one week period resulted in a significantly increased regional CBF response to stimulation (263). *Ellis et al.* found in rabbits that ARA induced a dose-dependent blood vessels dilation response of which the maximum was 100%. DHA itself had no effect on vessel diameter, but reduced the dilation produced by ARA (19). It may be hypothesized that intake of combined ARA and DHA could neutralize the cerebrovascular reactivity.

It is well known that obese or overweight patients have a reduced CBF (18, 264-266). In the current study however, HFHC-diet feeding increased CBF levels, but not the vasoactivity in later life. Moreover, HFHC diet was found to decrease the number blood vessels in the cortex, thalamus and hippocampus, which indicates that a HFHC diet is able to affect cerebrovascular integrity. Other studies revealed that exposure to a high fat diet could induce cerebrovascular remodeling (267-269). It should be taken into account that the blood vessel walls in the relatively mild ApoE3*Leiden obese mouse model are not severely damaged yet and may still be able to adapt to the HFHC induced changes. To elucidate these findings, future research should focus on the role of LCPUFAs in endothelial function, atherosclerotic plaque development and blood pressure in mildly obese and more pronounced obese rodent models in critical periods of development. Overall, we demonstrated that a HFHC-diet provokes adaptations in the cerebrovascular integrity and CBF. Furthermore, dietary ARA&DHA intake in early life is able to counteract these adaptations.

Cognition, brain structure and function

We did not find ARA&DHA effects on cognition after eight weeks of dietary intake. In line with this, no significant differences were found by *Lim et al.* in the results of the maze-learning tests between Crj:CD-1 mice fed DHA diets for a period of one or two weeks (270). However, after a 12 week period of DHA-supplementation, mice required less time to reach the maze exit and strayed into blind alleys fewer times (270). These results suggest that it may take time before learning abilities improve after the incorporation of DHA into the neuronal membranes (270). Mice fed an ARA&DHA-diet showed an increased functional connectivity. In line with our findings, a study of *Grayson et al.* demonstrated that cortical modular organization in monkeys supplemented with DHA resembled a healthy human brain (271). Furthermore, individuals with low levels of dietary LCPUFAs had decreased functional connectivity within the early visual pathway and throughout

higher-order associative cortex and showed impairment of distributed cortical networks (271). These findings emphasize that LCPUFAs modulate brain structure, i.e. functional connectivity.

The ARA&DHA fed mice used a more specific, advanced, and hippocampal dependent cognitive search strategy later in life. This finding implies that dietary ARA&DHA intake can improve hippocampal function. Short exposure to a high fat and sucrose diet has been shown to impair hippocampal dependent place recognition, but not the object recognition (272, 273). HFD exposure in rats impaired long-term spatial reference memory in the Morris water maze, without affecting acquisition or short-term memory (274). Accordingly, LCPUFAs and high fat diets seem to specifically modulate spatial memory.

We revealed an increased structural loss of gray matter in later life due to HFHC exposure. Moreover, ARA&DHA dietary intake protected against neuronal loss. Human studies revealed that obese children had significant regional gray matter reduction in middle temporal gyrus, thalami, pre and postcentral gyrus, and cerebellum (92, 275). DHA plays a crucial role in maintaining cortical neuronal integrity (276). In healthy subjects supplementation with DHA, EPA and vitamin E significantly attenuated gray matter volume loss in the hippocampus and temporal brain regions (277). These findings emphasize the ability of a HFHC diet and LCPUFAs to affect gray matter structure and volume. *Kullman et al.* reported that obese individuals showed a decreased functional connectivity strength in the left insular cortex (278). The insular cortex is considered to be the primary gustatory cortex (279), as it represents an important relay of the neural circuitry, essential in food intake, connecting the hypothalamus, orbitofrontal cortex and limbic system. We showed that functional connectivity was reduced in mice fed HFHC-diet, especially within the hippocampus, motor, and somatosensory cortex. These findings indicate that a HFHC-diet induces impairments within the gray matter neuronal structure and functional connectivity in specific brain regions. Dietary ARA&DHA intake in early life is able to counteract these HFHC diet-induced detrimental effects on brain structure and function in later life.

The study further emphasizes the importance of LCPUFAs as dietary components to counteract detrimental effects related to overweight and obesity, and that early life LCPUFAs support cognitive flexibility during later life challenges. Sensitive imaging techniques are essential to investigate and demonstrate the subtle detrimental adaptations induced by a HFHC diet in a mildly obese rodent model. Future research should focus on interactions of dietary components, optimal supplementation period and the vulnerability within different periods of life.

3

BUTYRATE RESTORES HFD INDUCED ADAPTATIONS IN BRAIN FUNCTION AND METABOLISM IN MID-ADULT OBESE MICE

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Abstract

Midlife obesity affects cognition and increases risk of developing dementia. Recent data suggest that intake of the short chain fatty acid butyrate could improve memory function, and may protect against diet-induced obesity by reducing bodyweight and adiposity.

We examined the impact of a high fat diet (HFD) followed by intervention with 5% (w/w) dietary butyrate on metabolism, microbiota, brain function and structure in the low-density-lipoprotein receptor knockout (LDLr-/-) mouse model in mid- and late life.

In mid-adult mice, 15 weeks of HFD induced adiposity, liver fibrosis and neuroinflammation, increased systolic blood pressure and decreased cerebral blood flow, and functional connectivity assessed with neuroimaging. The subsequent two months butyrate intervention restored these detrimental effects to chow fed control levels. Both HFD and butyrate intervention decreased variance in fecal microbiota composition. In late adult mice, HFD showed similar detrimental effects and decreased cerebral white and gray matter integrity, whereas butyrate intervention attenuated only metabolic parameters.

HFD induces detrimental effects in mid- and late adult mice, which can be attenuated by butyrate intervention. These findings are consistent with reported associations between midlife obesity and cognitive impairment and dementia in humans. We suggest that butyrate may have potential in prevention and treatment of midlife obesity.

Introduction

Obesity-related diseases are becoming one of the major health-care problems of the twenty-first century (1). Obesity is associated with structural brain changes, cognitive impairment and neurodegenerative diseases, besides the well-known metabolic complications (2, 3). Particularly in midlife, it has been reported that obesity can affect cognitive functioning and increase the risk of developing dementia (3). It has been hypothesized that obesity may exaggerate aging processes (280). Multiple rodent studies provided indications for adverse effects of obesity on cognitive functioning in aged mice (281, 282). Therefore, novel approaches related to weight management in aging are urgently required.

The potency of short chain fatty acids such as butyrate has been demonstrated in treatment of obesity and obesity related disease conditions in recent animal studies (20). Butyrate is a natural product of bacterial fermentation of mainly undigested plant polysaccharides and resistant starch in the colon. It has anti-inflammatory properties and is involved in the regulation of liver lipogenesis, adipocyte differentiation and energy intake (23). In addition, butyrate is associated with associative learning and memory functions in severe Alzheimer's disease mouse models via mediating histone acetylation (283). Obese humans have a decreased concentration of butyrate-producing bacteria (24).

In this study, we evaluated the effect of butyrate on microbiota, brain structure and function, and key metabolic organs in context of high fat diet (HFD)-induced obesity. LDLr-/-Leiden (LDLr-/-) mice were assessed *in vivo* and *ex vivo* experiments in two age groups, mid- (3-6 months old (m.o.)) and late (6-12 m.o.) adult mice. The LDLr-/- Leiden mouse model was chosen for this study because of its high sensitivity to develop obesity and obesity-associated metabolic and vascular complications in multiple organs when fed HFD with 45% fat content (190, 191, 284, 285), and subsequently we think these complications can detrimentally affect brain function. Our results provide evidence that butyrate can attenuate HFD induced impairment during mid- and late adulthood, whereas HFD induced alternations in brain function can only be restored in mid-adulthood. Overall, this study elucidates the effect of diet in the maintenance of metabolic and cognitive flexibility during life.

Materials and Methods

Animals and diets

Male LDLr-/- Leiden mice originated from the SPF breeding stock at TNO (TNO Metabolic Health Research, Leiden, the Netherlands), and were housed in individually ventilated cages in conventional animal rooms in the preclinical imaging centre (PRIME) at the central animal laboratory, Radboudumc Nijmegen, the Netherlands (ad libitum access to acidified tap water and food; relative humidity 50–60%, temperature 21°C, light cycle 7 a.m.–7 p.m. and a maximum of 5 mice per cage). The animal experiments were approved by an independent institutional ethical committee on animal care and experimentation (Zeist, The Netherlands), approval number DEC3682 containing a statistical power analysis to minimize the number of animals. This study is performed and reported according to ARRIVE guidelines (286).

To examine age differences, we assessed a cohort of 30 LDLr-/- mice of three m.o. and a second cohort of six m.o. mice representing mid- and late adulthood, respectively. Both cohorts consisted of three diet groups: Chow (33.0% kcal protein, 58.0% carbohydrates and 9.0% kcal fat, Sniff R/M-H diet V1530, Sniff Spezialdiäten GmbH, Soest, Germany), HFD (20.0% kcal protein, 35.0% carbohydrates and 45.0% kcal fat, D12451, Research Diets Inc, New Brunswick, USA) and HFD enriched with 5% (w/w) butyrate (HFDB). The dietary compositions are presented in supplementary Table 1. The first cohort, representing mid-adulthood, consisted of a group receiving a standardized chow diet from birth until the end of the study, a second group received a HFD at three months of age and a third group switched to a HFD after three months as well, and at seven m.o. the HFD was enriched with butyrate for two months (Fig. 1A). The second cohort representing late adulthood was subdivided in the same manner, only switched to a HFD at six m.o. and received at ten m.o. a butyrate intervention (Fig. 1B). Mice were examined for diet-induced changes in microbiome, metabolism, cognition, brain structure and function (Fig. 1).

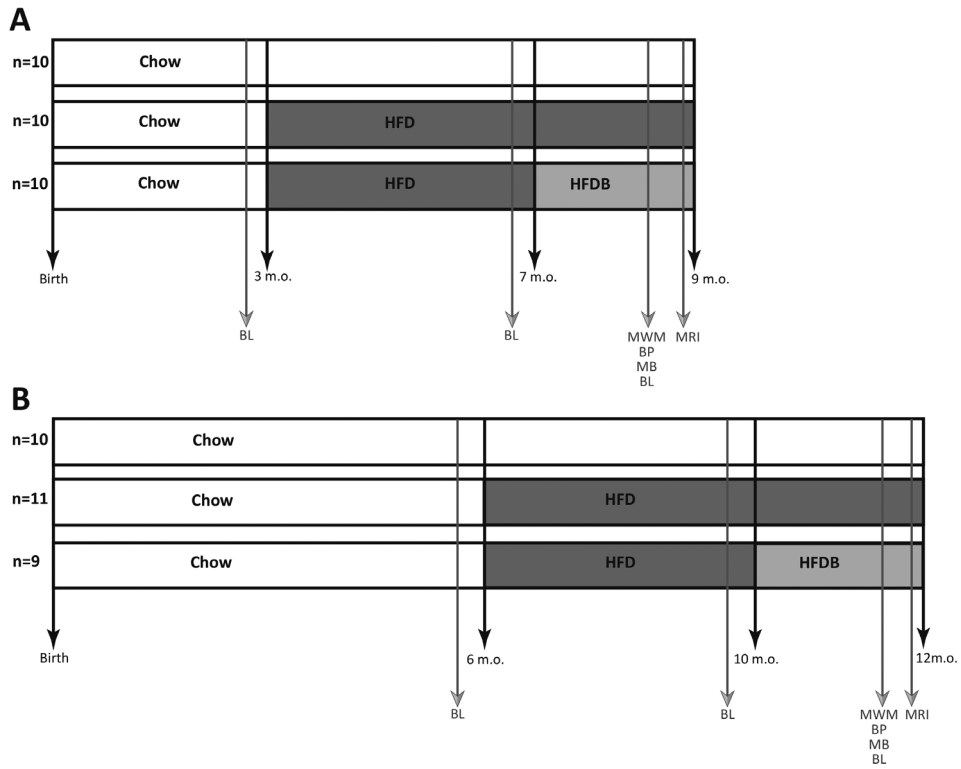

Figure 1. Study design. *A) Mid-adulthood, high fat diet (HFD) feeding started at 3 months of age and when 7 m.o. the butyrate intervention started (HFD enriched with butyrate (HFDB)). Cognitive and MRI experiments were performed at 9 months of age. B) Late adulthood, exposure of the HFD started at 6 m.o., and at 10 months of age the butyrate intervention started. Late adult mice were assessed in the cognitive and MRI experiments at 12 months of age. Individual body weight and food intake (at cage level) were monitored over time. Blood samples were taken after 5 hours of fasting (8 a.m.–1 p.m.). At the end of the experiment, all mice were anesthetized and sacrificed by transcardial perfusion with 0.1M phosphate-buffered saline (PBS). Thereafter organs were harvested and used for immunohisto- and biochemical experiments. MB= collect faeces for microbiome analyses; BL= blood sample collection. MWM= assessed in Morris water maze; BP=blood pressure measurements; MRI= examined with magnetic resonance imaging for resting state fMRI (rsfMRI), arterial spin labelling (ASL) and diffusion tensor imaging (DTI). HFD=high fat diet; HFDB=high fat diet enriched with butyrate; m.o.=months old; n= group size.*

Plasma analyses

Total plasma cholesterol, insulin and triglyceride levels were measured using standardized ELISA kits (202). Glucose levels were assessed using the OneTouch Ultra glucometer (Lifescan Benelux, Beerse, Belgium).

Fecal sample collection and DNA extraction
Fecal samples were collected at the end of the study and frozen immediately at −80°C. Samples were mechanically homogenized and genomic DNA was isolated using the AGOWA mag Mini kit (DNA Isolation Kit, AGOWA, Berlin, Germany) according to the manufacturer's instructions.

Metagenomic sequencing
A fragment of the 16S rRNA gene (~270 bp), spanning the V4 hypervariable regions, was PCR amplified using F515/R806 primers within 30 cycles (287). Purified PCR products were paired-end sequenced on the Illumina MiSeq platform (Illumina, Eindhoven, The Netherlands). The sequence data was processed with Mothur v.1.33.2 (288) and grouped using Minimum Entropy Decomposition (MED) algorithm that clusters 16S rRNA gene amplicons in a sensitive manner (289). Sequences were taxonomically classified by the RDP-II Naive Bayesian Classifier using a 60% confidence threshold. Community profiles were compared by Bray–Curtis dissimilarity and Weighted Unifrac clustering of MED abundance (290). Sequences were normalized to 10000 sequences per samples. Population-level comparison of MED abundance between samples collected was performed using the Metastats tool (291).

Morris water maze
The Morris water maze (MWM) was assessed to examine long term memory and spatial learning abilities. We performed and analyzed the MWM according to standardized protocols previously described (292).

Blood pressure measurements
A computerized and warmed tail-cuff plethysmography device was used to measure systolic blood pressure (SBP; IITC Life Scientific Instruments, Woodland Hill, CA). The mice were habituated to the restrainer and acclimatized to the complete procedure. Six SBP-measurements per trained mouse were averaged to obtain the mean SBP.

MRI-experiments
MRI measurements were performed with a 11.7 T Biospec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) which operated with a Paravision 6.0 software platform. Isoflurane (Nicholas Primal (I) Ltd, London, United Kingdom) was used for anaesthesia (3.5% for induction for 2 minutes and approximately 1.8% for maintenance in a mixture of oxygen and medical air (1:2)). We used standardized protocols for these MRI measurements published by *Zerbi et al.* (226), and we assessed functional connectivity (FC) (resting state fMRI), regional cerebral blood flow (arterial spin labeling (ASL)) and gray and white matter

integrity (diffusion tensor imaging (DTI)). Imaging parameters are represented in supplementary table 2. Mice were excluded from further MRI analysis in case of positioning artefacts.

(Immuno)histochemistry

Mice were sacrificed via transcardial perfusion with 0.1 M phosphate-buffered saline (PBS), and organs harvested e.g. brain, liver and fat depots. Organs used in (immuno)histochemical experiments were post-fixed in 4% paraformaldehyde for 24 hours. 5 μm thick paraffin-embedded cross-sections of liver tissue were stained with hematoxylin and eosin. NAFLD was scored blinded using a general scoring system for rodent models according to an established protocol (293). Ionized calcium binding adapter molecule-1 (IBA-1) antibody was used to detect activated microglia (goat anti-IBA-1 (1:2500; Abcam, ab5076)) as indicator for neuroinflammation in 30-μm thick coronal brain sections. We used a previously described standardized protocol for immunohistochemistry and its quantification (213).

Quantitative real-time polymerase chain reaction

The snap-frozen dissected hippocampus of the right hemisphere was used for the analysis of mRNA levels using quatitative real-time polymerase chain reaction (qRT-PCR) in duplo according to a previously described protocol (213). Forward and reverse primer sequences are represented in supplementary table 3. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a reference gene serving as a control for general cell activity.

Statistical analysis

A random and double-blind selection procedure was used throughout the experiment. The results are expressed as means ± standard error of mean (SEM). Means were analyzed using repeated measures or multivariate analysis of variance (ANOVAs) with Bonferroni correction for multiple testing in, SPSS20 (IBM SPSS Statistics 20, IBM Corporation, Armonk, New York, USA). Microbiota correlation analyses were performed using Pearson correlations. Supplementary table 4 provides the P and F values of the found significant effects.

Results

Butyrate restored HFD induced metabolic adaptations in mid- and late adult mice

In the mid-adult cohort (9 m.o.), body weight of the HFD mice increased over time and was 30% higher ($P<0.001$) compared to Chow mice at the end of the experiment. A two months butyrate intervention in mid-adult mice on HFD did not significantly reduce bodyweight of the HFD fed mice (16%; $P=0.11$). However,

a two months butyrate intervention in late adult mice on HFD resulted in a body weight loss of 23% (P<0.001) compared to HFD fed mice. In this late adult cohort (12 m.o.), body weight of the HFD fed mice was 35% higher (P<0.001) compared to chow fed mice. (Fig. 2 and Supplementary table 4). Food intake was monitored per cage (n=2 or 3) in grams, and subsequent calculated into kilo calorie intake per day per mouse. No significant differences were found between the three dietary interventions in intake (kcal) over time indicating that the energy intake was the same for all diet groups (Fig. 2 and Supplementary table 4).
A HFD increased the cholesterol and triglycerides plasma levels in mid- and late adult mice compared to Chow. The subsequent butyrate intervention lowered the plasma levels of cholesterol and triglycerides in both mid- and late adult mice. No diet effects were found in glucose plasma levels in age cohorts. In mid-adulthood, plasma insulin levels were slightly increased by HFD. Butyrate intervention restored insulin levels compared to solely a HFD which were even below Chow insulin levels, which was more pronounced in late adulthood.
Liver, epididymal, inguinal and omental fat depots was weighed after sacrifice. Liver weight, omental and inguinal fat depot weight were increased in HFD fed mice both at age cohorts compared to chow fed mice. Subsequent butyrate intervention restored these metabolic adaptations (Supplementary table 4).

Butyrate recovered hepatic steatosis induced by HFD feeding in mid- and late adult mice

A HFD increased the NAFLD as hepatic steatosis and hypertrophy were increased in mid- and late adult mice. In late adult mice a HFD increased the percentage of fibrosis. A butyrate intervention restored these observed changes in the liver as it decreased hypertrophy, fibrosis and macrovesicular steatosis (Fig.2). In addition, inflammatory aggregates in the liver were increased in late adult mice fed a HFD (Supplementary Fig. 1)**.**

Butyrate restored HFD induced spatial memory impairment in mid-adult obesogenic mice

Long term memory and spatial learning were assessed in the MWM. In mid-adulthood, HFDB mice had a shorter latency to reach the platform than HFD mice (Supplementary Fig. 2A). In the first 30 seconds of the probe trial, mid-adult HFD mice were in the former platform NE-quadrant for a shorter time period, compared to HFDB mice (Supplementary Fig. 2C), indicating a diminished spatial memory. The swim paths of all mice were classified to assess the degree of used hippocampus dependent search strategies. Mid-adult mice fed HFD showed less hippocampus-dependent search strategies than mid-adult mice fed chow (Supplementary Fig. 2D). In late adulthood, none of these parameters (acquisition, probe NE duration and search strategy) revealed a significant diet effect indicating that diets are no longer effective in late adulthood concerning spatial memory.

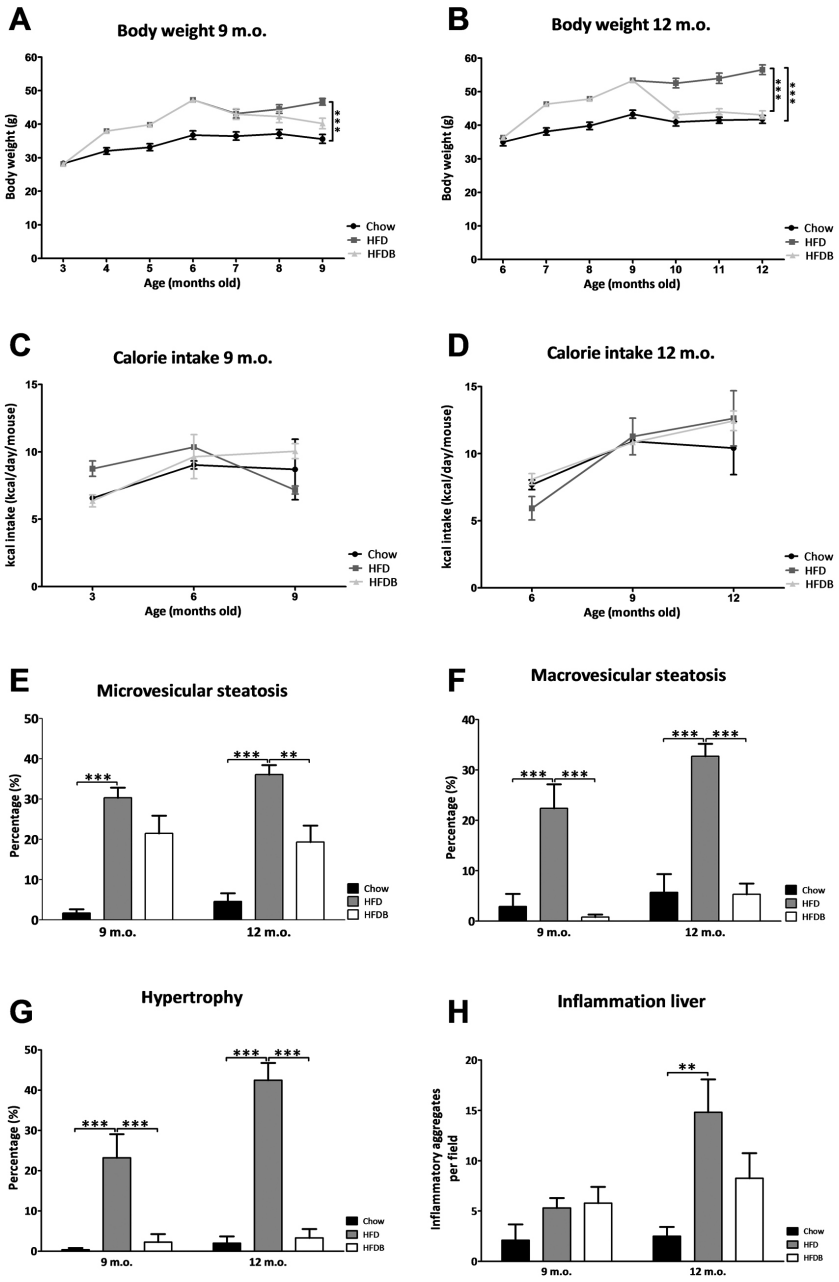

Figure 2. Body weight, calorie intake and liver tissue analysis. *A) Body weight in mid adult (9 m.o.) mice at a HFD. A HFD enriched with butyrate (HFDB) started at 7 months of age. B) Body weight in late adult mice (12 m.o.). HFDB started at 9 months of age. C) Calorie intake in mid-adulthood. D) Calorie intake in late adulthood. E) Percentage of microvesicular steatosis. F) Percentage of macrovesicular steatosis. G) Percentage of hypertrophy of hepatic cells. H) Percentage of fibrosis within liver tissue. 9 m.o.= mid-adult. 12 m.o.= late adult. Data are presented as mean ± SEM. *P<0.050.*

Butyrate restored HFD induced SBP and CBF in mid-adulthood

LDLr-/- mice had an increased SBP between 120-150 mmHg whereas a normal SBP-range of an adult mouse is around 80-100 mmHg (294). Mid-adult mice fed a HFD had an increased SBP compared to chow fed mice (Fig. 3A). Mid-adult HFD mice had a decreased CBF within the hippocampus and thalamus, compared to both Chow and HFDB mice. Butyrate intervention restored the CBF to control levels in the hippocampus and thalamus (Fig. 3C-D).

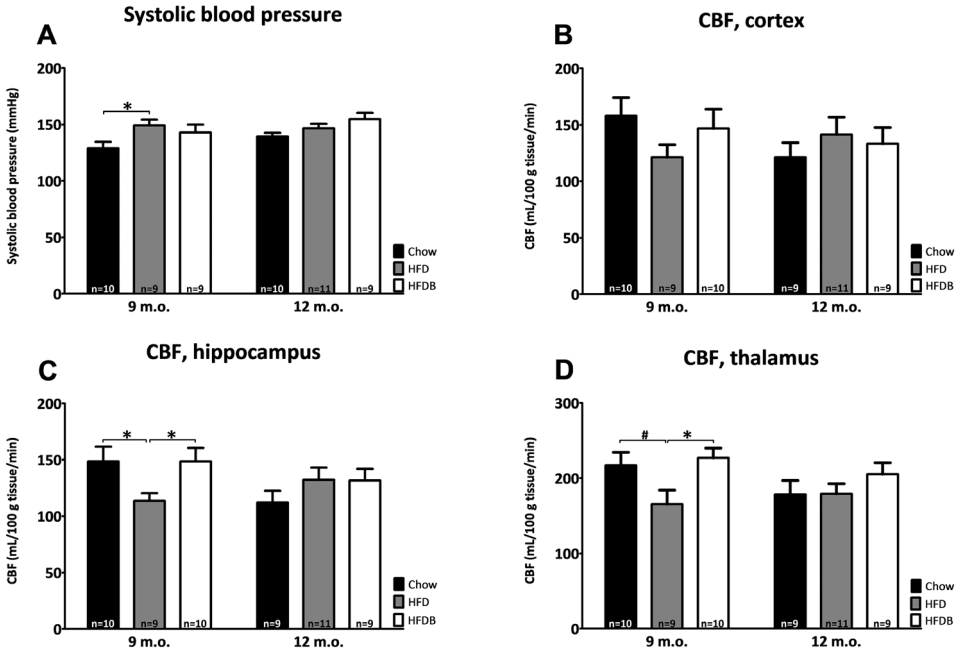

Figure 3. Systolic blood pressure and cerebral blood flow in the cortex, hippocampus and thalamus (A-D). *A) Mean systolic blood pressure (SBP) in mmHg in mid- (9 m.o.) and late (12 m.o.) adult LDLr-/- mice. Six measurements were used to calculate the mean SBP per mouse. B, C, D) Mean cerebral blood flow (CBF) right and left cortex (B), hippocampus (C) and thalamus (D) in mL/100 g tissue/min. Data are presented in mean ± SEM. *P<0.050.*

Butyrate restored functional connectivity in mid-adult HFD fed mice

Mid-adult HFD mice had a decreased FC within the somatosensory cortex and hippocampus compared to Chow mice (Fig. 4A-C). Butyrate intervention was able to restore impairments in FC induced by HFD feeding in mid-adult mice (Fig. 4D-F). FC results of late-adult mice are represented in supplementary figure 3. DTI was assessed to indicate diet effects on gray and white matter integrity. No diet effects were found in the mid-adult mice, whereas late adult mice showed a reduced white and gray matter integrity (Supplementary Fig. 4).

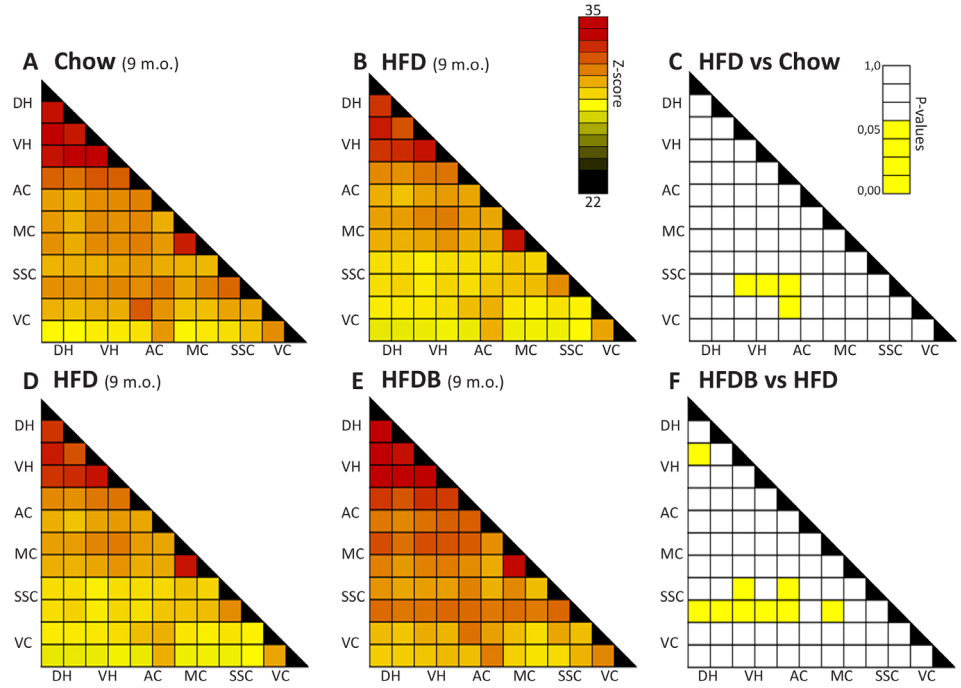

Figure 4. resting state fMRI (rsfMRI) of mid-adult mice (9 m.o.). *A, B, D and E) Total correlation matrixes of the Chow (A), HFD (B & D) and HFDB (E) diet group of mid-adult mice (9 m.o.). The selected brain regions (dorsal hippocampus (DH), ventral hippocampus (VH), auditory cortex (AC), motor cortex (MC), somatosensory cortex (SSC) and visual cortex (VC)) are subdivided in left hemisphere region (first row) and right hemisphere region (second row). In general the DH and VH are strongly bilaterally connected, and AC, MC and SSC are highly interconnected. A higher Z-score (red) indicates a stronger functional connectivity (FC). C & F) Statistical analyses of FC to reveal a significant diet effect, which are represented as a matrix. Yellow: P<0.050. More specifically, mid-adult mice on HFD showed reduced FC within the somatosensory cortex and hippocampus when compared to mice on Chow diet (A-C). In addition, butyrate intervention was able to restore the loss of FC mainly within the hippocampus and somatosensory cortex in mid-adult mice (D-F). Chow, n=10; HFD, n=10 and HFDB, n=9.*

Butyrate decreased neuroinflammation in mid-adult HFD fed mice

IBA-1 immunohistochemistry indicated the level of activated microglia as measure of neuroinflammation. In the hippocampus and thalamus, the number of activated microglia was increased in mid-adult HFD mice compared to the chow fed mice (Fig. 5E-G). A butyrate intervention was able to decrease the number of activated microglia in the total hippocampus, cornu ammonis 1 (CA1) and thalamus (Fig. 5E-G). qRT-PCRs for tumor necrosis factor-α, interleukin-6 and -1β to analyze inflammatory levels in the hippocampus revealed no significant effects. We analyzed mRNA levels of synaptic markers: synaptophysin and postsynaptic density

protein-95 (PSD-95). In the hippocampus of mid-adult mice synaptophysin mRNA levels were higher in HFD than in chow and HFDB fed mice. No diet effects were found for PSD95 mRNA levels (Supplementary table 4 and Fig. 5).

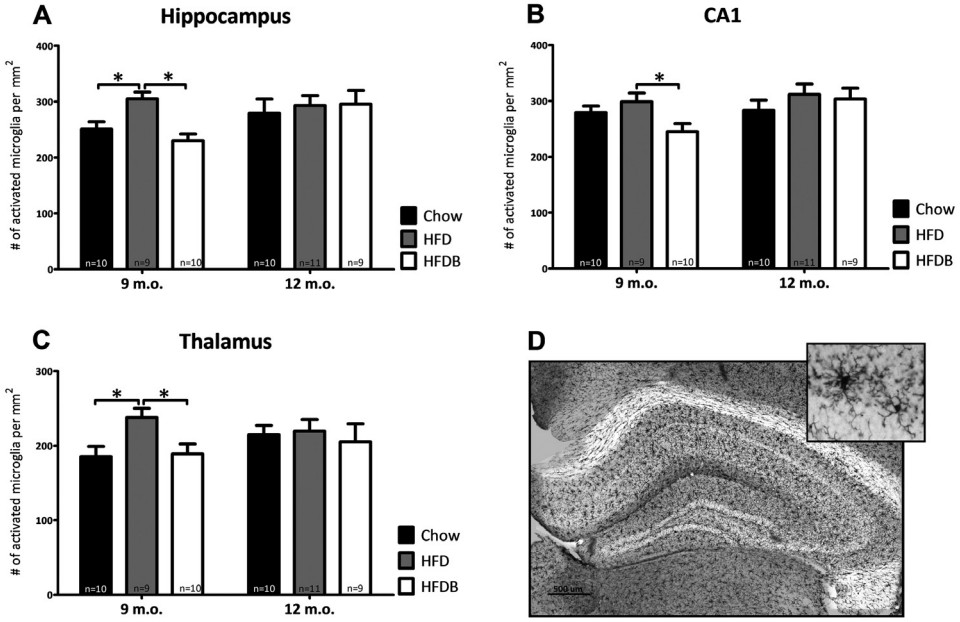

Figure 5. Quantification of activated microglia as visualized by IBA-1 immunhistochemistry (E-H). *E-G) Number of activated microglia within the hippocampus, cornu ammonis 1 (CA1) and thalamus in mid- (9 m.o.) and late (12 m.o.) adult mice. H) Visualization (late adult HFDB) of IBA-1 immunohistochemistry, positive for activated microglia, within the hippocampus at 5x and 40x magnification. Data are presented as mean ± SEM. *P<0.050.*

HFD caused shifts in gut microbiota composition

We observed dramatic differences in microbiota at genus level between chow and HFD fed mice based on Bray-Curtis dissimilarity index regardless of age (Supplementary Fig. 6A). Butyrate intervention in both mid- as well as late adult mice resulted in distinct changes in microbiota composition (Supplementary Fig. 6B-C).

Butyrate-related changes in microbiota are associated with liver fibrosis and neuroinflammation

Pearson correlation analyses were used to identify a possible correlation of key pathological features (i.e. liver fibrosis in late adult mice and neuroinflammation in mid-adult mice) with the observed changes in fecal microbiota. Twelve bacterial genera showed significant correlation with liver fibrosis (Corr. c oefficient cut-off 0.4; P<0.05; Fig. 6A-C). The abundance of *Clostridium Sensu Stricto* (R=0.59; P=0.003), *Bifidobacterium* (R=0.57; P=0.005) and *Enterorhabdus* (R=0.56; P=0.006) showed a positive correlation while *Clostridium XIVb* (R=-0.50; P=0.01) showed a

negative correlation with liver fibrosis. Thirteen bacterial genera showed significant correlation with neuroinflammation (Corr. coefficient cut-off 0.4; P<0.05; Fig. 6D-F). The abundance of *Dorea* (R=0.70; P<0.001), *Clostridium IV* (R=0.55; P=0.007) showed a positive correlation while *Clostridium Sensu Stricto* (R=0.-58; P=0.003) showed a negative correlation with neuroinflammation.

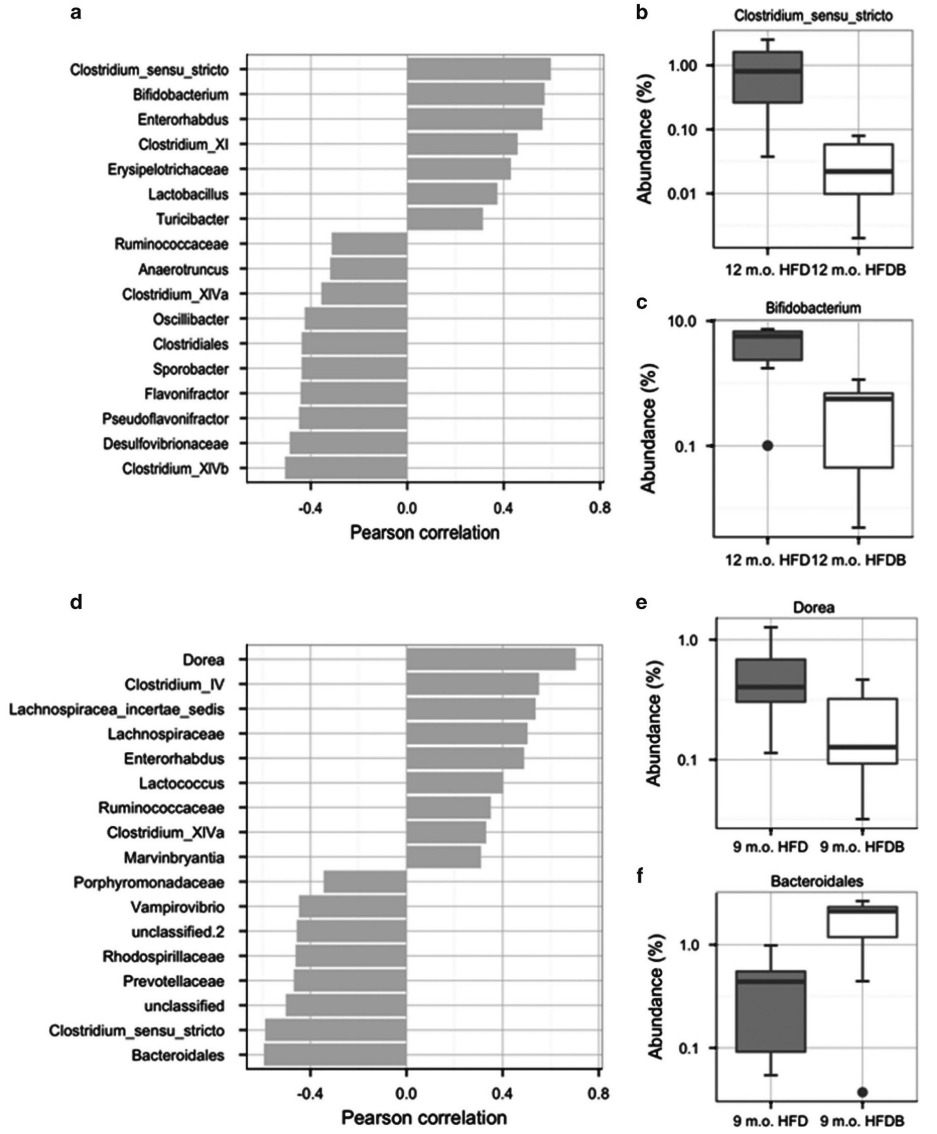

Figure 6. Fecal microbiota changes associated with liver fibrosis and neuroinflammation. *A) Pearson correlation analysis was performed between fecal microbiota at the genus level and pathological liver fibrosis in late adult mice. B-C) examples of two bacterial genera that are correlated with liver fibrosis. D) Pearson correlation analysis was performed between fecal microbiota at the genus level and level of neuroinflammation in mid-adult mice. E-F) examples of two bacterial genera that are correlated with neuroinflammation. Data are presented in mean ± SEM. *P<0.05.*

Discussion

In this study, we demonstrated that butyrate intervention is able to restore HFD induced changes in body weight, adiposity, liver pathology, cerebral blood flow, functional connectivity and neuroinflammation in mid-adulthood. In late adulthood, both HFD and butyrate did not affect brain function, which could be caused by irreversible aging processes in late adulthood e.g. in the vasculature.
HFD feeding increased adiposity and body weight in LDLr-/- mice in this study, and these findings are repeatedly confirmed in rodent research (21, 295, 296). A butyrate intervention of two months restored these changes, and likewise others found that butyrate is able to reduce adiposity and body weight in obese mice (21, 22). Others found an increased activity in butyrate fed mice at night (21). We did not observe an increased activity in butyrate fed mice, like distanced swum or walked in respectively the MWM or open field test (results not shown) during day time, and unfortunately we did not examine night-time activity. Butyrate is thought to be involved in lipid metabolism by regulating and slowing down intestinal fat transport (297). Understanding of the molecular mechanism is still incomplete, but short chain fatty acids (SCFAs), like butyrate, can contribute to fatty acid oxidation in multiple tissues and decrease fat storage in white adipose tissue as butyrate can induce a switch from lipid synthesis to utilization (298). Typically, this metabolic adjustment involves a downregulation of processes orchestrated by the peroxisome profilerator-activated receptor-γ (PPARγ) (299). An intake of butyrate affects the metabolic adaptations to obesogenic diet feeding via its stimulating effects on fatty acid oxidation and utilization. In future research physical activity levels and energy expenditure of these mice should be measured during day and night.

Obesity and non-alcoholic fatty liver disease (NAFLD) are marked by a state of inflammation initiated by HFD feeding (17). Moreover, average liver weight increased after HFD feeding in rats, and fat and cholesterol content in the liver was four to five times higher as the liver serves an alternative depot for free fatty acids (24). In mid- and late adult mice the liver weight, hepatic steatosis and hypertrophy was increased after six months of HFD feeding, and a butyrate intervention of two months was able to reverse these processes. A HFD induces a metabolic overload and thereby the metabolic pressure on the liver, resulting in alternative fat storage and NAFLD, whereas a butyrate intervention can restore metabolic pressure and adaptations.

An obesity-inducing diet diminishes hippocampal dependent search strategies and can generate cognitive deficits in rats (296). Reduced expression of synaptic proteins are suggested to be an underlying mechanism for this cognitive dysfunction (296). We found an increase in mRNA levels of the presynaptic marker synaptophysin

in mice on HFD, suggesting a possible mechanism to compensate a shortage of synaptophysin protein, and maybe the formation of presynapses. Butyrate intervention improved the spatial learning and memory performance in the Morris water maze, and preserved presynaptic synaptophysin mRNA levels. It has been reported that butyrate administration improved associative memory and learning function (283, 300). Butyrate most likely affects cognitive function via enhancing histone acetylation by inhibiting histone deacetylase (HDAC) (283). Dynamic changes in histone acetylation have been linked to the genetic programming required for memory formation (283). For instance, sodium butyrate is a HDAC inhibitor and adaptations in histone acetylation lead to neuroprotective and neuroregenerative changes in animal models for neurodegenerative diseases (283, 300).

Obesity and in particular central obesity, has been consistently associated with hypertension in humans (301). In rodents, the SBP increased after 6 months on high fat and high sucrose diet in an obese mouse model (302). We found that a HFD increased the SBP in mid-adult LDLr-/- mice. An LDLr-/- mouse model is prone to develop narrowed blood vessels, arterial stiffness, atherosclerosis and hypertension especially when fed a HFD, because oxidized LDL particles accumulate in the intima layer of arteries (303). Late adult mice had a high SBP, independent of diet, suggesting that aging processes result in structural changes within the vasculature and reaching a maximum in SBP.

To this date, little research has been performed using neuroimaging on brain regions of the LDLr-/- mouse model. We gained novel insights using translational MRI techniques. Firstly, we revealed that cerebral blood flow (CBF) is decreased by a HFD in mid-adult LDLr-/- mice. In humans it is well established that overweight and obese individuals often have a decreased CBF, gray and white matter volumes (18, 89, 90). Secondly, we have demonstrated that a HFD decreases functional connectivity (FC) between the somatosensory cortex and hippocampus in mid-adult mice. We assume that the adaptations in CBF and FC could be provoked by arterial wall stiffening, increased blood pressure and the development of hypertension induced by HFD. It can affect the cerebrovasculature and modify CBF and via blood oxygen level-dependent (BOLD) signaling it may affect the FC. Butyrate neutralized the changes in CBF and improved the impaired FC induced by a HFD. Butyrate is defined as a classic anti-angiogenic agent, however in low concentrations it can stimulate angiogenesis (304) and thereby affect BOLD signaling. In late adult mice, we found no HFD effects on SBP, CBF, FC and spatial memory. An exact mechanism for this phenomenon is unknown, although it is possible that the vascular vessels and capillaries may already have reached a pronounced dysfunctional state due to aging that cannot be further aggravated by HFD. It is known that old LDLr-/-

mice on chow diet develop aortic plaques and atherosclerosis only due to aging (305). Moreover, the rate of development of atherosclerosis is more rapid between 10 and 14 months old LDLr-/- mice compared to older and younger LDLr-/- mice (306). Plasma cholesterol levels and vascular cell adhesion molecule 1 (VCAM-1) are possible contributors as their expression increases with age (306). We suggest that these aging processes induce a maximum, stationary, detrimental state within the vasculature. Furthermore this detrimental state cannot be further deteriorated by HFD nor improved by the dietary butyrate intervention tested herein. Mid-adult mice which have not reached this detrimental, potentially irreversible, state yet and may therefore be responsive to HFD as well as dietary intervention.

During mid-adulthood, HFD induced neuroinflammation within the thalamus and hippocampus. In line with this, a HFD increased the activation of microglia and inflammatory processes (295, 307). A butyrate intervention attenuated the inflammatory processes by decreasing the number of activated microglia within the thalamus, cortex and hippocampus, which confirmed earlier findings in literature (308). Butyrate has anti-inflammatory properties, and in more detail, butyrate activates free fatty acid receptor 3 (FFAR3) and 2 (FFAR2) (309-311) and therefore provide a link between butyrate and the central nervous system (CNS).

Analysis of the gut microbiome revealed pronounced changes in composition upon HFD feeding. These HFD induced changes were very consistent with observations made by others (312). The pronounced difference between chow and HFD composition made it difficult to discriminate the specific effects of butyrate from those of HFD on the microbiome. In line with observations made in obese humans which exhibit decreased levels of butyrate-producing bacteria compared to lean individuals (24), we found HFD induced in *Clostridium XIVa* abundancy. In an attempt to link the changes in microbiota with relevant pathophysiological endpoints, we tested whether specific genera were correlated to liver fibrosis and neuroinflammation. We observed a positive correlation between neuroinflammation and bacteria abundance of the genus *clostridia* (e.g. *Dorea, Clostridium IV, Lachnospiraceae, Lachnospiraceae incertae sedis,* and *Clostridium XIVa)* in fecal microbiota of mid-adult mice. A decreased abundance of these bacteria in HFDB fed mice correlated with a reduction in neuroinflammation as compared to HFD fed mice. Bacteria from the *clostridia* genus are known to be butyrate producing bacteria and are very abundant in the human colon (313). Our data suggest that dietary supplementation of butyrate in mid-adult mice could affect neuroinflammation despite a reduction of butyrate-producing bacteria in the fecal microbiota. Interestingly, this effect was less clear in the late adult mice in relation to hepatic fibrosis development where we observed both positive *(Clostridium sensu stricto, Clostridium XI)* as well as a negative correlation *(Clostridium XIVb,*

Clostridiales, Clostridium XIVa) of the butyrate-producing bacteria of the *Clostridia* genus. To our knowledge, these findings are novel however the data should be interpreted with caution because microbiome analysis and pathology have been performed at the same time point and microbiome changes may merely reflect metabolic adaptation but might not be causal to development of the end points. It is intriguing that the microbiota changes upon butyrate treatment differ in mid-adult and late adult mice and that the two age groups also develop different pathologic endpoints.

Conclusion

We hypothesize that midlife obesity increases the risk of cognitive impairment via mainly inflammatory responses and vascular damage. These findings emphasize the association between midlife obesity and cognitive impairment in humans (3). We report novel insights in the impact of diet-induced obesity and butyrate in a genetic mouse model on brain function and structure during mid- and late adulthood. In mid-adult humans, butyrate supplementation may serve as potential preventative for obesity and obesity-related diseases, and in the long-run as preventative for dementia.

Supplemental data:
https://www.nature.com/articles/ijo201752#supplementary-information

4

ADIPOSITY IS ASSOCIATED WITH VASCULAR AND VOLUMETRIC BRAIN OUTCOMES IN CEREBRAL SMALL VESSEL DISEASE.

THE RUN DMC STUDY

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[1] Authors contributed equally to the present work.

Abstract

Adiposity and changes in adiposity are associated with cerebrovascular diseases, such as cerebral small vessel disease (CSVD) and its sequelae, vascular cognitive impairments (VCI) and dementias. Body mass index (BMI), waist circumference (WC), and blood leptin and total adiponectin levels were associated with CSVD and brain volumetry in 503 adults age ≥50 years enrolled in the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC).

RUN DMC participants were followed for 9 years, from 2006 to 2015. BMI, WC, brain imaging and dementia diagnoses were evaluated at baseline and follow-up. Adipokines were measured at baseline. Brain imaging outcomes included CSVD components, white matter hyperintensities, lacunes and microbleeds; and gray and white matter, hippocampal, total brain, and intracranial volumes.

Cross-sectionally among men at baseline, higher BMI, WC and leptin were associated with lower gray matter and total brain volumes, and higher BMI and WC were associated with lower hippocampal volume. At follow-up 9 years later, cross-sectional analyses showed associations between higher BMI and lower gray matter volume in men, and that an obese WC (>102cm) was protective for presence of ≥1 lacune or ≥1 microbleed in men. In women, increasing BMI and overweight or obesity (BMI ≥25 kg/m^2 or WC >88 cm) were associated with ≥1 lacune.

Anthropometric and metabolic adiposity indicators were differentially associated with CSVD and brain volumes by sex. Adiposity is associated with a vascular-neurodegenerative spectrum that may influence and be influenced by brain pathologies associated with VCI and dementia.

Introduction

Approximately 50% of the global adult population is overweight or obese according to 2016 estimates, creating an obesity pandemic (1). Obesity during middle-age is associated with higher risk of several adult life cardiovascular risk factors and events including hypertension, hyperlipidemias, type 2 diabetes mellitus (T2DM), atherosclerosis, and myocardial infarction. These cardiovascular risk factors are subsequently associated with cerebrovascular events such as stroke and late-onset, sporadic dementias (315), in particular vascular cognitive impairments (VCI) and vascular forms of dementia (316).

The biological mechanisms linking obesity to VCI and dementias and underlying cerebrovascular and neuro-pathologies are not well understood. However, one potential mechanism is that obesity represents higher amounts of adipose tissue, which contribute to alterations in the peripheral and cerebral circulation, which may be more pronounced with aging (317). In the brain, this translates to decreased cerebral blood flow and cerebral small vessel disease (CSVD), which is considered the major vascular contributor to dementia (318, 319). While CSVD cannot be diagnosed *in vivo,* it is operationalized by the presence of brain MRI markers including white matter hyperintensities (WMH), lacunes and microbleeds.

Adipose tissue is the largest endocrine organ in the human body (77). Anthropometric measurements such as body mass index (BMI) and waist circumference (WC) are commonly used to estimate total and central adiposity, respectively. BMI grossly reflects total adult adiposity (5), whereas WC reflects the amount of highly bioactive visceral adipose tissue that surrounds internal organs (320). Secretory products of adipose tissue exhibit vascular and metabolic effects, and influence brain structure and function (77, 112, 321).
We examined the associations of anthropometric and metabolic measures of adiposity with MRI markers of CSVD (WMH, lacunes and microbleeds) and brain volumetry (gray and white matter, hippocampus and total brain). Given known sex differences in total and central adiposity, we stratified all analyses by sex. Identifying and understanding potential associations between potentially modifiable adiposity and CSVD, both of which increase risk for cognitive impairments and dementias, is important as this could facilitate interventions to reduce adverse effects of excess adiposity on public and personal health.

Methods

Study population

The RUN DMC study prospectively investigates vascular and other factors associated with CSVD and CSVD progression among a baseline sample of 503 adults, aged 50 to 85 years, with CSVD. Participants were recruited from consecutive patients referred to the department of Neurology of the Radboud university medical centre between October 2002 and November 2006, because they presented with symptoms of CSVD. These symptoms were acute, such as transient ischemic attack (TIA) or lacunar syndromes, but also subacute manifestations such as cognitive and motor (gait) disturbances (322). Patients were included based on age and the presence of CSVD on neuroimaging (WMH and/or lacunar infarcts) (322). Patients who were eligible because of a lacunar syndrome were included >6 months after the event to avoid acute effects on outcomes.

Exclusion criteria were: (a) dementia; (b) parkinsonism; (c) intracranial hemorrhage; (d) life expectancy <6 months; (e) intracranial space-occupying lesion; (f) psychiatric or other disease interfering with cognitive testing or ability to be followed up; (g) recent or current use of acetylcholinesterase inhibitors, neuroleptic agents, L-dopa or dopamine agonists or antagonists; (h) non-CSVD WMH (e.g. multiple sclerosis); (i) prominent visual or hearing impairment; (j) language barrier; or (k) MRI contraindications, e.g., claustrophobia.

Follow-up of participants after 9 years occurred among those who were alive and agreed to participate. Baseline participants were invited to follow-up via mail, with a follow-up telephone call to schedule their visit at our research center. During their follow-up visit, a subset of baseline assessments were re-administered, including a dementia assessment. All tests at baseline and follow-up were performed by two trained Neurology residents physicians.

All participants provided written informed consent. The study was approved by the Medical Review Ethics Committee region Arnhem-Nijmegen.

Structured interview

A structured interview interview was done, which included on demographics, lifestyle, vascular risk factors, and current medication use. *Demographics and lifestyle* included education (classified using 7 categories, ranging from primary school to an academic degree), marital status, living conditions, and lifestyle habits (alcohol consumption, smoking). *Vascular risk factors and cardiovascular disease* included history of hypertension, T2DM, atrial fibrillation, TIA, stroke, myocardial infarction, coronary artery bypass graft, per-cutaneous transluminal coronary angiography, aortic prothesis, vascular prothesis, carotid endartectomy, and migraine. Family history of myocardial infarction, cerebrovascular disease and

diabetes mellitus was recorded. *Current medication* use was classified according to the anatomical therapeutic chemical (ATC) classification system (323).

Physical examination

Anthropometric measurements included body mass index (BMI), calculated as clinically measured body weight divided by body height squared (kg/m^2). Maximal waist circumference (WC) in centimeters (cm) was measured without a shirt, in a standing position, between the lowest rib and the iliac crest at the end of usual expiration.

Blood pressure measurements included systolic and diastolic pressures obtained in the sitting position. The average of three repeated measurements was used.

Blood measures at baseline were collected after a 12-hour, overnight fast, and serum aliquots were stored at −80°C. A lipid panel comprised of total cholesterol, HDL, LDL and triglyceride, was measured. Total serum leptin and adiponectin levels were determined using human leptin and adiponectin enzyme-linked immunosorbent assays (ELISA) (Laboratory Medicine, Radboud University Medical Center, Nijmegen, The Netherlands).

Magnetic Resonance Imaging protocol

MRI images were acquired on a 1.5T MRI scanner (2006: Siemens (Munich, Germany), Magnetom Sonata, and 2015: Siemens, Magnetom Avanto) using the same head coil at both time points. The scanning protocol included 3D T1 magnetization-prepared rapid gradient echo (MPRAGE) imaging (voxel size 1.0x1.0x1.0 mm); and fluid-attenuated inversion recovery (FLAIR) pulse sequences (2006: voxel size 0.5x0.5x5.0 mm, interslice gap 1.0 mm, and 2015: voxel size 0.5x0.5x2.5 mm; interslice gap 0.5 mm). To minimize effects of changes in FLAIR sequence, we re-sliced follow-up FLAIR images to match slice thickness of baseline images using linear interpolation (324).

Brain volumetry outcomes

MRI outcomes analyses have been described in detail (324). In brief, gray matter, white matter, hippocampal and cerebrospinal fluid (CSF) volumes were calculated using SPM12 (fil.ion.ucl.ac.uk/spm/) unified segmentation routines on the T1 MPRAGE images (325). All images were visually checked for co-registration and segmentation artefacts. Gray matter volumes (GMV), white matter volumes (WMV), and CSF volumes were computed by summing all voxels belonging to that tissue class, multiplied by voxel volume in milliliters. Intracranial volume (ICV) was determined by summing GMV, WMV, and CSF volume; and total brain volume (TBV) by summing GMV and WMV. To account for interscan effects, we corrected for differences in ICV between baseline and follow-up. All volumes were normalized to baseline ICV to account for head size.

CSVD outcomes

CSVD was rated using STRIVE criteria for vascular changes on neuroimaging (326). WMH volumes were calculated using a semiautomatic WMH segmentation method (327). Segmentations were visually checked for segmentation errors by one trained rater, blinded to clinical data. The Fazekas scale was used to categorize baseline WMH severity (328).

Number and location of lacunes and microbleeds were rated manually on FLAIR/T1-weighted MRI scans according to the STRIVE criteria by 2 trained raters blinded to clinical data. Lacunes were defined as hypo-intense areas >2 mm and ≤15 mm on FLAIR and T1, ruling out enlarged perivascular spaces (≤2 mm, except around the anterior commissure, where perivascular spaces can be large) and infraputaminal pseudolacunes (329). Microbleeds were defined as small (<10 mm diameter), homogeneous, round foci of low signal intensity on T2* weighted images (330). Microbleed number per hemisphere was counted. Lesions were not considered microbleeds if they were symmetric hypointensities in the globus pallidus, likely calcifications or iron deposits (330). Inter-rater and intra-rater reliabilities were excellent (324). Incident lacunes and microbleeds during the follow-up period were identified (324).

Dementia assessment

Dementia screening was performed at 9-year follow-up and has been described in detail (325). The mini–mental state examination (MMSE)(331) was the initial screening instrument of global cognition. A follow-up MMSE score <26 or a decline of ≥3 points from baseline was considered screening positive. Those who screened positive based on these criteria were subsequently examined for the presence of dementia at the Radboud Alzheimer Center Nijmegen, The Netherlands. If a participant refused additional examinations at the Alzheimer Center, a consensus panel consisting of a neurologist, clinical neuropsychologist, and a geriatrician made the dementia diagnosis. This panel reviewed all available neuropsychological (322) and brain imaging information, which included a) difference in neuropsychological performance between baseline and follow-up; b) outcome of the Mini International Neuropsychiatric Interview MINI (332); c) the follow-up MRI scan and/or baseline MRI-scan to determine dementia subtype; and d) medical record review. In addition, participants' general practitioners and medical specialists were contacted regarding cognitive status. Cognitive tests were interpreted with consideration for age, level of education, and interference with daily living confirmed by family or caregiver (333). The dementia diagnosis was based on the diagnostic and statistical manual of mental disorders (DSM) IV criteria (334). Dementia onset was established as the date when clinical symptoms were consistent with the diagnosis (335).

Statistical analyses

Baseline and follow-up cross-sectional analyses included descriptive analyses of all primary adiposity exposures and brain outcomes for the entire sample and by sex. Means and standard deviations (SD) were computed for continuous variables. Due to skewed distributions, leptin, adiponectin and WMH were natural log transformed. As leptin resistance is often observed in obesity, we estimated leptin resistance by calculating the leptin to BMI (leptin:BMI) ratio (336). Pearson correlation analyses were conducted among adiposity measures. T-tests were used for means comparisons. Chi-square analyses were conducted for categorical variables.

Multivariable linear and logistic regression models were used to examine cross-sectional associations between adiposity measures and CSVD and volumetric brain outcomes at both time points, 2006 and 2015. Regression models were run separately for each adiposity measure as an independent predictor. Adiposity measures included BMI and WC in 2006 and 2015; and leptin and total adiponectin in 2006 only. Brain imaging outcomes included CSVD components and brain volumes. Logistic regression analyses were used to predict the presence *versus* absence of CSVD components. Odds ratios (OR) and 95% Confidence Intervals (CI) were calculated. WMH, GMV, WMV, TBV and hippocampal volume (HV) were considered as continuous outcome variables in linear regression analyses. Beta-coefficients (b) and 95% CI were calculated.

Given that CSVD and more severe change in body weight and BMI increases risk for dementia (337), we also examined change in anthropometric measures (BMI and WC) from 2006-2015 in association with MRI markers of CSVD and brain volumes measured in 2015. In analyses of anthropometric change, since no participant presents with the same body weight or BMI at two visits and 'any change' may not be informative, we categorized the sample by an *a priori* defined 'substantial' degree of change, either ≥5% and ≥10% increase or decrease from baseline body weight and BMI.

Selection of covariates originated from a pool of variables that were potentially biologically relevant and/or reported in the literature as CSVD risk and protective factors. Our pool consisted of age; sex; educational attainment (≤ primary education *versus* > primary education); cigarette smoking in pack-years (calculated as number of packs of cigarettes smoked per day multiplied by number of years smoked); alcohol intake (units per day); clinically relevant depressive symptom burden (≥16 on the center of epidemiologic studies on depression scale (CES-D) or current depression medication use); MMSE score; use of glucose lowering medications; T2DM (self-reported or T2DM medication use); hypertension (blood pressure

≥140/90 mmHg or antihypertensive medication use); blood lipid levels; and hypercholesterolemia (cholesterol ≥ 6.2mM or lipid-lowering drug use). From this pool, we systematically determined which ones to include in multivariable-adjusted regression models by evaluating each variable in age-adjusted regression models in association with brain MRI outcomes. If the variable was associated with an outcome at P≤0.05, the variable was included as a covariate in the final regression model. As a result, age, sex, education (≤ primary school versus > primary school), cigarette smoking (ever/never), and T2DM (yes/no) were included. In analyses involving 2015 brain outcomes, presence of dementia and baseline WMH severity based on the Fazekas scale, were also included as covariates. Results for linear and logistic multivariable analyses were considered significant using two-tailed tests, P<0.05. SPSS, version 22.0 (IBM Corporation, Armonk, New York, USA), was used to conduct data analyses.

Results

Baseline characteristics of the RUN DMC baseline study population (N=503) are reported in Table 1. Mean age of participants at baseline was 65.6 ± 8.8 years, and at follow-up, 71.3 ± 7.9 years. Differences in baseline characteristics between participants and those who died during follow-up are also presented in Table 1. Compared to surviving participants, those who died during follow-up were older; had a lower MMSE score; had higher average WC; had higher prevalence of central obesity using sex-specific cut points; were more likely to use lipid- glucose- and blood pressure-lowering medications; and were more likely to have T2DM. Those who died also evidenced lower baseline TBV, GMV, WMV and HV; a higher median volume of WMH; and were more likely to have a Fazekas score of moderate or severe (*versus* none or mild) or possess ≥ 1 lacune at baseline. Thus, 'healthier' surviving participants were observed in 2015.

Adiposity indicators, CSVD markers, and brain volumes

Increasing BMI and WC, as well as total and central obesity were associated with lower GMV and TBV in all male participants cross-sectionally at average baseline age, 66 years (Table 2). Higher serum leptin levels were associated with lower GMV and TBV in the total sample of men and women. Increasing BMI and WC were also associated with lower HV in men (Table 2). Interestingly, a higher estimate of leptin resistance (leptin:BMI), was also associated with lower GMV and TBV, particularly in men. At follow-up 9 years later, average age 71 years, cross-sectional analyses showed increasing BMI associated with lower GMV; and higher WC with lower HV, particularly in men (Table 3).

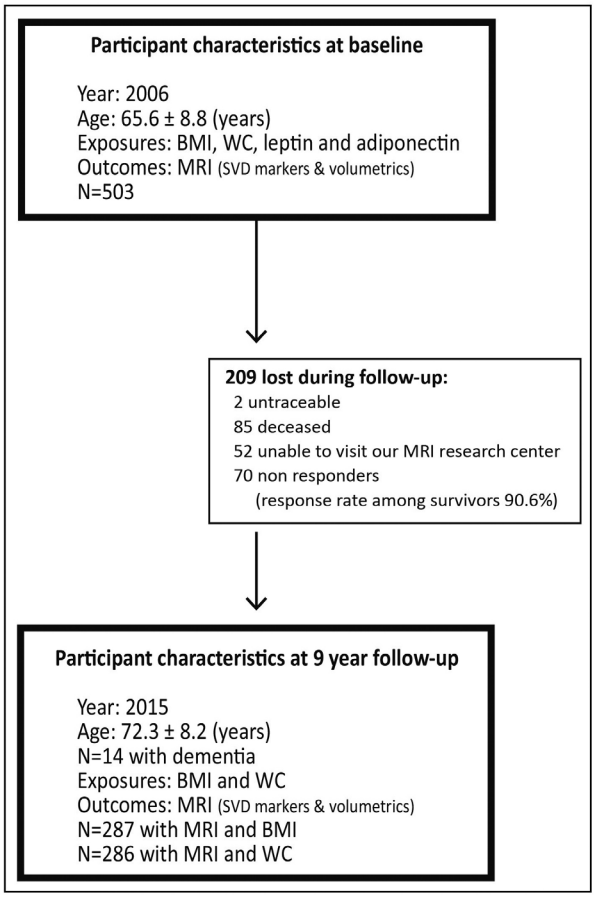

Figure 1. RUN DMC study design

In relation to CSVD outcomes, cross-sectionally at average age 66 years, leptin was protective in men. In particular, the highest quartile of leptin, compared to the lowest quartile, was associated with lower WMH volumes and lower odds of ≥1 lacune. Cross-sectional analyses at 9-year follow-up, showed that an obese WC lowered the odds of microbleeds and lacunes in men (Table 4). However, in women, increasing BMI, being overweight or obese based on BMI, or having an obese WC were associated with the presence of ≥1 lacune (Table 4).

Longitudinal analyses of baseline adiposity measures in association with CSVD and brain volumetry outcomes at follow-up 9 years later showed no associations (data not shown). Similarly, evaluation of 5% and 10% change in BMI and body weight over 9 years, showed no association with follow-up CSVD or brain volumetry outcomes (data not shown).

	Baseline 2006			Deaths during follow-up			Follow-up 2015		
	All	Men	Women	All	Men	Women	All	Men	Women
	N=503	N=284	N=219	N=85	N=47	N=38	N=287	N=164	N=123
Demographics									
Age (years), mean ± SD	65.6 ± 8.8	65.7 ± 8.8	65.5 ± 8.9	72.4 ± 8.1[c]	72.8 ± 6.6[c]	71.9 ± 9.7[c]	71.3 ± 7.9	71.3 ± 7.9	71.0 ± 7.4
Education <8 years, N (%)	49 (9.7)	26 (9.2)	23 (10.5)	13 (15.3)[c]	6 (12.8)	7 (18.4)[c]			
Depressive symptoms ≥16, N (%)	167 (33.2)	81 (28.5)	86 (39.3)[b]	50 (58.8)	20 (52.6)	19 (28.4)[b,c]			
MMSE score, mean ± SD	28.1 ± 1.6	28.1 ± 1.7	28.2 ± 1.6	27.3 ± 1.9[c]	27.1 ± 1.9[c]	27.6 ± 1.9[c]	27.5 ± 3.5	28.1 ± 2.0	28.3 ± 2.0
Dementia incidence, N (%)				23 (27.1)	9 (19.1)	14 (36.8)	14 (4.9)	10 (6.1)	4 (3.3)
Vascular risk factors [a]									
Alcohol consumption (units per week), mean ± SD	7.9 ± 9.3	10.8 ± 10.3	4.1 ± 6.2[b]	8.4 ± 10.8	12.9 ± 12.5	2.8 ± 3.8*			
Smoking (ever), N (% yes)	353 (70.2)	236 (83.1)	117 (53.4)[b]	66 (77.6)	45 (95.7)	21 (55.3)*			
Lipid lowering drugs, N (% yes)	237 (47.1)	143 (50.4)	94 (42.9)	51 (60.0)[c]	30 (63.8)	21 (55.3)			
Glucose lowering drugs, N (% yes)	66 (13.1)	45 (15.8)	21 (9.6)[b]	22(25.9)[c]	14 (29.8)[c]	8 (21.1)[b,c]			
Hypertension, N (% yes)	369 (73.4)	208 (73.2)	161 (73.5)	73 (85.9)[c]	41 (87.2)	32 (84.2)			
Diabetes mellitus, N (% yes)	75 (14.9)	50 (17.6)	25 (11.4)[b]	23 (25.9)[c]	15 (31.9)[c]	8 (21.1)[b,c]			
Adiposity indicators									
BMI (kg/m^2), mean ± SD	27.2 ± 4.1	27.3 ± 3.8	27.0 ± 4.5	26.9 ± 4.3	27.0 ± 4.5	26.8 ± 4.5	27.2 ± 4.0	27.2 ± 3.6	26.9 ± 4.3
Healthy (<25.0 kg/m^2), N (%)	152 (30.2)	78 (27.5)	74 (33.8)	29 (34.1)	78 (27.5)	74 (33.8)	90 (31.3)	48 (29.3)	42 (34.1)
Overweight (25.0-29.9 kg/m^2), N (%)	226 (44.9)	139 (48.9)	87 (39.7)	35 (41.2)	139 (48.9)	87 (39.7)	132 (45.9)	80 (48.8)	52 (42.3)
Obese (>30.0 kg/m^2), N (%)	125 (24.9)	67 (23.6)	58 (26.5)	21 (24.7)	67 (23.6)	58 (26.5)	65 (22.6)	36 (21.9)	29 (23.6)
Change in BMI, 2015-2006 (%), mean ± SD							0.44 ± 8.3	0.11 ± 6.8	0.88 ± 9.5
Waist circumference (WC, cm), mean ± SD	96.0 ± 12.2	99.9 ± 10.6	91.0 ± 12.4[b]	97.0 ± 12.4	101.4 ± 10.2	91.6 ± 12.7[b]	99.1 ± 12.4	102.4 ± 11.2	93.5 ± 12.0[b]
Healthy WC (men < 102 cm, women < 88cm), N (%)	245 (48.7)	154 (54.2)	91 (41.6)[b]	36 (57.6)	22 (46.8)	14 (36.8)[b]	122 (42.7)	86 (52.8)	36 (30.1)
Obese WC (men ≥ 102 cm, women ≥ 88cm), N (%)	258 (52.3)	130 (45.8)	128 (58.4)[b]	68 (54.8)	25 (53.2)	24 (63.2)[b]	164 (57.3)	78 (47.9)	86 (69.9)
Change in WC, 2015-2006 (%), mean ± SD							4.3 ± 8.7	3.8 ± 8.0	4.9 ± 9.3
Leptin (ng/mL), mean ± SD	23.4 ± 16.2	13.9 ± 10.5	35.7 ± 22.5[b]	26.4 ± 23.6	15.3 ± 11.4	39.8 ± 27.6[b]			
Leptin resistance (leptin:BMI)	0.8 ± 0.6	0.5 ± 0.3	1.2 ± 0.7[b]	0.9 ± 0.8	0.5 ± 0.4	1.4 ± 0.9[b]			
Adiponectin (ng/mL), mean ± SD	4.9 ± 3.3	3.6 ± 2.2	6.5 ± 3.8[b]	6.2 ± 4.5	3.9 ± 2.2	9.0 ± 5.2[b]			
Brain volumes									
Total brain volume (mL), mean ± SD	1060.9 ± 80.1	1046.0 ± 81.0	1080.0 ± 74.9[b]	573.0 ± 52.7[c]	559.9 ± 47.9[c]	589.2 ± 54.4[c]	1043.7 ± 78.7	1025.5 ± 80.8	1068.0 ± 69.0[b]
Gray matter volume (mL), mean ± SD	606.2 ± 52.6	593.2 ± 51.4	623.1 ± 49.2[b]	427.3 ± 45.9[c]	420.8 ± 53.3[c]	435.4 ± 33.9[c]	597.6 ± 50.6	584.67 ± 47.7	617.4 ± 47.4[b]
White matter volume (mL), mean ± SD	454.7 ± 46.0	452.9 ± 48.5	457.1 ± 42.5	7.1 ± 1.0[c]	6.9 ± 0.9[c]	7.3 ± 1.0[c]	443.8 ± 46.1	439.1 ± 50.6	449.8 ± 39.5
Hippocampal volume (mL), mean ± SD	6.7 ± 1.0	7.3 ± 1.0	7.9 ± 1.0[b]				7.4 ± 1.1	7.1 ± 1.0	7.3 ± 1.0[b]
CSVD markers									
WMH (mL), median (IQR)	3.6 (10.0)	3.2 (9.6)	4.1 (10.8)[b]	11.5 (15.0)[c]	11.2 (14.4)[c]	12.6 (16.4)[c]	4.7 (10.3)	3.8 (7.6)	5.67 (13.7)
Microbleeds, N (% any)	83 (16.5)	50 (17.6)	33 (15.1)	18 (21.2)	13 (27.7)[c]	5 (13.2)	70 (24.4)	38 (23.2)	32 (26.0)
Lacunes, N (% any)	132 (26.2)	86 (30.3)	46 (21.0)[b]	43 (50.6)[c]	27 (57.4)[c]	16 (42.1)[b,c]	79 (27.5)	50 (30.4)	29 (23.6)

Table 1. *Baseline and follow-up characteristics of participants, and baseline characteristics of those died during follow-up in the RUN DMC . [a]Vascular risk factors assessed at baseline only. [b]Significant at P<0.05 men versus women. [c]Significant P<0.05 participants who died during follow up versus baseline. WMH: White Matter Hyperintensities. CSVD: cerebral small vessel disease. IQR: interquartile range.*

Baseline 2006		Total Brain Volume		Gray Matter Volume		White Mater Volume		Hippocampal Volume	
	N	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Continuous adiposity measures									
BMI (kg/m²)	503	-0.05(-0.13- 0.03)	0.21	**-0.16(-0.24- -0.09)**	**0.00**	0.07(-0.01-0.16)	0.11	**-0.08(-0.16- -0.01)**	**0.04**
BMI (kg/m²), men	284	**-0.12(-0.23-0.04)**	**0.00**	**-0.28(-0.41- -0.18)**	**0.00**	0.08(-0.04-0.22)	0.19	**-0.12(-0.26- 0.01)**	**0.04**
BMI (kg/m²), women	219	0.01 (-0.08- 0.09)	0.90	-0.04 (-0.14- -0.06)	0.48	0.06(-0.06-0.17)	0.35	-0.06(-0.17- 0.06)	0.38
WC (cm)	503	-0.07(-0.16-0.02)	0.11	**-0.18(-0.26- -0.10)**	**0.00**	0.05(-0.04-0.14)	0.31	**-0.11(-0.20- -0.02)**	**0.01**
WC (cm), men	284	**-0.13(-0.25- -0.06)**	**0.00**	**-0.25(-0.40- -0.16)**	**0.00**	0.03(-0.11-0.18)	0.31	-0.10(-0.26- 0.02)	0.08
WC (cm), women	219	-0.02 (-0.11- 0.08)	0.76	-0.09 (-0.19- 0.03)	0.16	0.07(-0.06-0.19)	0.32	-0.11(-0.23- 0.02)	0.10
Leptin (ng/mL)	503	**-0.18(-0.26- -0.07)**	**0.00**	**-0.13(-0.22- -0.04)**	**0.00**	0.04(-0.06-0.15)	0.42	-0.05(-0.14-0.05)	0.36
Leptin (ng/mL), men	284	-0.07(-0.19- 0.02)	0.11	**-0.16(-0.32- -0.06)**	**0.00**	0.05(-0.09-0.21)	0.43	-0.07(-0.22-0.06)	0.27
Leptin (ng/mL), women	219	-0.02 (-0.13- 0.10)	0.76	-0.05 (-0.19- -0.08)	0.44	0.02(-0.13-0.18)	0.74	-0.00(-0.15-0.16)	0.97
Adiponectin (ng/mL)[b]	503	-0.07(-0.16-0.02)	0.11	0.02(-0.06-0.10)	0.65	0.04(-0.06-0.13)	0.44	0.01(-0.08-0.10)	0.78
Adiponectin (ng/mL), men	284	0.04(-0.04-0.14)	0.28	0.06(-0.05-0.17)	0.30	0.03(-0.09-0.16)	0.62	0.05(-0.07-0.17)	0.44
Adiponectin (ng/mL), women	219	0.01(-0.10-0.12)	0.89	-0.03(-0.17-0.10)	0.60	0.04(-0.10-0.20)	0.53	-0.02(-0.19-0.13)	0.73
Leptin resistance	503	**-0.16(-0.24- -0.08)**	**0.00**	**-0.11(-0.18- -0.03)**	**0.01**	0.01(-0.08-0.09)	0.92	-0.03(-0.12-0.06)	0.51
Leptin:BMI, men	284	-0.05(-0.14-0.03)	0.20	**-0.13(-0.24- -0.02)**	**0.02**	0.04(-0.08-0.16)	0.48	-0.07(-0.19-0.05)	0.27
Leptin:BMI, women	219	-0.07(-0.16-0.04)	0.22	-0.08(-0.19-0.05)	0.24	-0.04(-0.18-0.09)	0.54	0.04(-0.10-0.17)	0.61
Categorical adiposity measures									
BMI (kg/m²)									
Total sample									
Healthy: ≤24.9[a]	152	1.0		1.0		1.0		1.0	
Overweight & Obese: ≥25.0	351	-0.03(-0.11- 0.05)	0.53	**-0.09(-0.17- -0.02)**	**0.02**	0.06(-0.03-0.15)	0.17	-0.03(-0.12-0.05)	0.44
Men vs women									
Normal weight: ≤24.9[a]	78/74	1.0		1.0		1.0		1.0	
Overweight & Obese: men ≥25.0	206	-0.07(-0.16- 0.01)	0.08	**-0.16(-0.27- -0.05)**	**0.00**	0.04(-0.08-0.17)	0.46	-0.12(-0.27-0.05)	0.17
Overweight & Obese: women ≥25.0	145	0.03 (-0.06- 0.12)	0.56	-0.03 (-0.11- -0.11)	0.96	0.08(-0.05-0.19)	0.25	-0.06(-0.22-0.12)	0.54
WC (cm)									
Total sample									
Healthy WC: men ≤102 & women ≤88[a]	245	1.0		1.0		1.0		1.0	
Obese WC: men >102, women >88	258	**-0.10(-0.18- -0.02)**	**0.01**	**-0.15(-0.23- -0.07)**	**0.00**	0.03(-0.06-0.12)	0.53	**-0.10(-0.19- -0.01)**	**0.02**
Men vs women									
Small WC: men ≤102 & women ≤88[a]	154/91	1.0		1.0		1.0		1.0	
Large WC: men >102	130	**-0.12(-0.20- -0.03)**	**0.01**	**-0.15(-0.23- -0.07)**	**0.00**	0.02(-0.10-0.14)	0.53	**-0.13(-0.25- -0.02)**	**0.03**
Large WC: women >88	128	-0.03 (-0.12- 0.07)	0.60	-0.06 (-0.17- 0.06)	0.32	0.03(-0.10-0.16)	0.62	-0.07(-0.20-0.07)	0.32
Leptin (ng/mL)									
Total sample									
Q1: men ≤ 7.4, women ≤19.6[a]	127	1.0		1.0		1.0		1.0	
Q4: men >21.7, women > 52.6	126	**-0.21(-0.25- -0.07)**	**0.00**	**-0.17(-0.20- -0.04)**	**0.00**	0.02(-0.08- 0.11)	0.74	-0.11(-0.18 – 0.02)	0.10
Men vs women									
Q1: men ≤ 7.4, women ≤19.6[a]	72/55	1.0		1.0		1.0		1.0	
Q4: men >21.7	71	-0.09(-0.16- 0.03)	0.17	**-0.19(-0.24- -0.02)**	**0.02**	0.05(-0.10- 0.17)	0.74	-0.16(-0.25-0.01)	0.07
Q4: women > 52.6	55	-0.12(-0.20- 0.04)	0.18	-0.16(-0.23- 0.02)	0.10	-0.04(-0.20-0.13)	0.68	-0.02(-0.19-0.15)	0.82

Table 2. Linear regression models estimating baseline cross-sectional associations between adiposity exposures and brain volumes in RUN DMC. *Linear regression models for gray matter, white matter and hippocampal volumes were adjusted for age, education, sex, smoking, and type 2 diabetes mellitus (T2DM). N: number of participants. Seven participants were excluded in the hippocampal analyses due to imaging artifacts (motion). β: beta, standardized regression coefficient. Continuous measures of total brain volume, gray matter volume, white matter volume and hippocampal volume (mL). Continuous levels of leptin and adiponectin are categorized in quartiles, and the lowest quartile (Q1) was compared to the highest quartile (Q4). [a]Referent category. [b]No significant associations were observed for adiponectin quartiles (data not shown).*

Follow-up 2015		Total brain volume		Gray matter volume		White matter volume		Hippocampal volume	
	N	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Continuous adiposity measures									
BMI (kg/m²)	287	-0.04(-0.12- 0.05)	0.38	**-0.12(-0.23- -0.02)**	**0.02**	0.06(-0.05-0.18)	0.29	-0.02(-0.11- -0.08)	0.71
BMI (kg/m²), men	164	-0.05(-0.18- 0.07)	0.41	**-0.14(-0.29- -0.03)**	**0.04**	0.05(-0.11-0.24)	0.45	0.05(-0.09-0.19)	0.48
BMI (kg/m²), women	123	-0.03(-0.14-0.09)	0.69	-0.09(-0.22-0.07)	0.30	0.05(-0.10-0.19)	0.56	-0.08(-0.21-0.07)	0.32
WC (cm)	286	-0.05(-0.14- 0.04)	0.26	**-0.13(-0.24- -0.01)**	**0.03**	0.05(-0.08-0.18)	0.46	-0.03(-0.14- -0.07)	0.56
WC (cm), men	163	-0.04(-0.18- 0.08)	0.44	-0.11(-0.26- 0.04)	0.16	0.02(-0.16-0.21)	0.80	0.06(-0.09-0.21)	0.42
WC (cm), women	123	-0.05(-0.18- 0.08)	0.44	-0.16(-0.31- 0.01)	0.07	-0.12(-0.28-0.03)	0.11	-0.12(-0.28-0.03)	0.11
Categorical adiposity measures									
BMI (kg/m²)									
Total sample									
Normal weight: ≤24.9 [a]	88	1.0		1.0		1.0		1.0	
Overweight & Obese: ≥25.0	199	-0.03(-0.08- 0.08)	0.94	-0.09(-0.19- -0.01)	0.08	0.08(-0.04-0.19)	0.19	0.00(-0.09-0.10)	0.93
Men vs women									
Normal weight: men/women ≤24.9[a]	47/41	1.0		1.0		1.0		1.0	
Overweight & Obese: men ≥25.0	117	0.07(-0.11- 0.12)	0.89	-0.09(-0.19- -0.01)	0.08	0.08(-0.05-0.26)	0.17	0.03(-0.10-0.15)	0.70
Overweight & Obese: women ≥25.0	82	-0.01(-0.13-0.11)	0.86			0.05(-0.11-0.19)	0.95	-0.01(-0.15-0.14)	0.95
WC (cm)									
Total sample									
Small WC: men ≤102, women ≤88[a]	122	1.0		1.0		1.0		1.0	
Large WC: men >102, women >88	164	0.03(-0.16- 0.18)	0.94	-0.08(-0.37- -0.06)	0.18	0.07(-0.10-0.38)	0.26	-0.05(-0.10- 0.29)	0.34
Men vs women									
Small WC: men ≤102, women ≤88[a]	85/37	1.0		1.0		1.0		1.0	
Large WC: men >102	78	0.01(-0.11- 0.12)	0.89	-0.08(-0.37- -0.06)	0.18	0.08(-0.14-0.50)	0.28	**0.14(-0.01-0.52)**	**0.04**
Large WC: women >88	86	-0.01(-0.13-0.11)	0.86	-0.06(-0.20-0.09)	0.46	0.07(-0.22-0.48)	0.47	-0.06(-0.47-0.19)	0.41

Table 3. Linear regression models estimating cross-sectional associations between anthropometric measures and brain volumes at 9-year follow-up in RUN DMC. *Linear regression models for gray matter volume, white matte volume and hippocampal were adjusted for: age, education, sex, smoking, type 2 diabetes mellitus (T2DM), WMH severity, and dementia incidence. N: number of participants. Five participants were excluded in the hippocampal analyses due to imaging artifacts (motion). β: beta, standardized regression coefficient. Continuous measures of total brain volume, gray matter volume, white matter volume and hippocampal volume (mL). [a]Referent category.*

Follow-up 2015		WMH[b]			≥ 1 Microbleeds[c]		≥ 1 Lacunes[c]	
	N	β (95% CI)	p	N	OR (95% CI)	p	OR (95% CI)	p
Continuous adiposity measures								
BMI (kg/m²)	287	0.05(-0.05-0.15)	0.31	276	0.95(0.88-1.03)	0.20	1.03(0.95-1.11)	0.51
BMI (kg/m²), men	164	0.05(-0.10-0.19)	0.50	161	0.90(0.80-1.01)	0.09	0.93(0.83-1.04)	0.19
BMI (kg/m²), women	123	0.05(-0.10-0.20)	0.53	116	0.99(0.90-1.11)	0.97	**1.17(1.03-1.32)**	**0.01**
WC (cm)	286	-0.05(-0.06-0.16)	0.39	274	0.98(0.95-1.01)	0.15	1.01(0.97-1.03)	0.69
WC (cm), men	163	0.03(-0.13-0.19)	0.69	157	0.96(0.92-1.0)	0.05	0.97(0.94-1.01)	0.19
WC (cm), women	123	0.04(-0.13-0.21)	0.61	116	0.99(0.95-1.03)	0.60	1.04(0.99-1.09)	0.07
Categorical adiposity measures								
BMI (kg/m²)								
Men vs women								
Normal weight: men/women ≤24.9[a]	47/41	1.0		47/40	1.0		1.0	
Overweight & Obese: men ≥25.0	117	0.04(-0.11-0.18)	0.62	113	0.51(0.23-1.15)	0.11	0.60(0.26-1.40)	0.23
Overweight & Obese: women ≥25.0	82	0.10(-0.06-0.25)	0.21	76	1.73(0.64-4.62)	0.28	**6.97(1.68-28.97)**	**0.01**
WC (cm)								
Men vs women								
Small WC: men ≤102, women ≤88[a]	85/37	1.0		85/36	1.0		1.0	
Large WC: men >102	78	0.03(-0.21- 0.35)	0.63	73	**0.42(0.18-0.97)**	**0.04**	**0.32(0.14-0.75)**	**0.01**
Large WC: women >88	86	0.07(-0.23-0.51)	0.45	80	0.79(0.30-2.08)	0.64	**4.66(1.11-19.42)**	**0.03**

Table 4. Linear and logistic regression models estimating cross-sectional associations between adiposity exposures and cerebral Small Vessel Disease outcomes at 9-year follow-up in RUN DMC. *Linear regression models for WMH were adjusted for: age, education, sex, smoking, type 2 diabetes mellitus (T2DM), CSVD severity, and dementia incidence. Logistic regression models for incident microbleeds were adjusted for: age, education, sex, WMH severity, and dementia incidence; and incident lacunes for: age, education, sex, smoking, hypertension, T2DM, CSVD severity, and dementia incidence. N: number of participants. Nine participants were excluded from microbleeds analyses due to imaging artifacts (motion). WMH: White Matter Hyperintensities. [a]Referent category. [b]Continuous measures of WMH (mL), [c]presence of any microbleeds or lacunes. CSVD: cerebral Small Vessel Disease. β: beta, standardized regression coefficient. OR: Odds Ratio. (95% CI): 95% Confidence Interval.*

Discussion

To our knowledge, these data comprise a first report suggesting sex differences underlying associations of anthropometric and metabolic adiposity measures with cerebrovascular and neurodegenerative events in a clinical cohort of CSVD patients. Overweight and obesity, as well as higher leptin levels, in men with CSVD are associated with neurodegeneration, particularly in their 60s. However, among men with CSVD surviving into their 70s, higher adiposity is associated with fewer cerebrovascular markers. In contrast, women with CSVD and higher levels of adiposity in their 70s are more likely to experience lacunes and evidence no associations with neurodegeneration. These data also illustrate the nuances of following a healthier group of surviving participants with a clinically diagnosed cerebrovascular phenotype. Most observed associations occurred among men at two cross-sectional time points, at average age 66 years, and 9 years later at average age 71 years, with adverse associations with adiposity diminishing with survivorship.

Associations of higher BMI and WC with underlying cerebrovascular pathologies such as white matter hyperintensities (WMH) (338), cerebral microbleeds (339) and lacunes (340) are sparsely reported in the literature. Most data suggest higher levels of vascular risk factors, including higher BMI and central obesity or visceral adipose, to be associated with higher cerebrovascular risk (318, 341). In addition, a J-shaped BMI curve, i.e. both low and high BMI, is associated with higher risk of intracerebral haemorrhage (342). Higher WC has been associated with CSVD components, notably characterized by microbleeds, lacunes, and WMH in Korean samples (318, 339).

In addition to considering cerebrovascular components of CSVD, we also evaluated neurodegeneration. Cross-sectional associations between higher anthropometric measurements and lower TBV, GMV and HV is comparable to others reporting that higher levels of anthropometric measures are associated with a lower TBV, GMV and HV (57, 343, 344). Higher BMI has been associated with lower brain volumes, particularly GMV, in adults (345, 346), including those with metabolic syndrome (347). Higher BMI and lower GMV have also been associated with lower cognitive function (80); as has central obesity (89). Visceral adipose tissue (highly correlated with WC) measured via abdominal MRI, has been associated with lower HV in cognitively healthy elderly age 55-90 years (348). Despite the majority of studies reporting associations between higher adiposity and lower brain volumes, there are conflicting reports. For example, higher BMI was associated with higher HV in older adults with or at risk for cardiovascular disease (349).

Our findings suggest that first, higher levels of adiposity, classified as anthropometric overweight and obesity, may be related to earlier neurodegenerative outcomes, such as lower TBV and GMV, and later protection for lacunes and microbleeds in men. In contrast, among women, higher levels of adiposity are related to later occurrence of at least one marker of vascular damage, lacunes. This suggests a cerebrovascular-neurodegenerative spectrum and distinct temporality of brain outcomes associated with adipose tissue exposures that differ between men and women with CSVD. Second, these findings emphasize that adipose tissue may play endocrine, neuronal and vascular roles in brain health (77, 104, 350). These roles may be both cause and consequence of cerebrovascular and neurodegenerative pathologies associated with CSVD and/or its clinical symptoms. Third, these data underscore the profound influence of selective survival in longitudinal analyses of brain outcomes, and imply a role of brain reserve in survival, since those who died during the follow-up period had lower baseline brain volumes.

From the standpoint of metabolic adipose tissue, higher blood leptin levels and calculated leptin resistance are associated with lower TBV and GMV in older adults, particularly men with CSVD. This is somewhat novel, especially for older adults. Higher blood leptin levels have been associated with lower GMV in young adults (mean age 32.0 ± 1.0 years) (351). Among adolescents (mean age 15.3 ± 1.1 years) who gained body fat, there was observed greater GMV reduction in the putamen (352). Another study found higher leptin associated specifically with lower insula GMV among adults age 25-40 years who had a family history of obesity (353). Higher leptin levels are found in ischemic stroke patients (354). In contrast, among Japanese elderly, higher blood leptin levels were associated with higher region-specific GMV, including in the hippocampus (355). Higher leptin levels have also been shown to protect against age-related cognitive decline(355) and dementia (111) in older adults. Given that leptin signaling is dysregulated in degenerating hippocampal neurons, potentially indicating neuronal leptin resistance in Alzheimer's disease patients (356), inconsistent cross-sectional leptin associations with brain outcomes, cognition and dementias in older men may be obscured. Leptin may play a pivotal role in an aging fat-brain axis, as well as be a critical marker of aging-related changes in satiety, glucose homeostasis, inflammatory processes, vascular function, and memory formation (112, 117, 321, 357-360).

Sex differences in total and central obesity is a known phenomenon (361, 362). Differential storage of white adipose tissue is influenced by sex hormones, as well as fat depot-specific release of hormones and cytokines (363). Larger WC, a marker of visceral adipose tissue and denoting a more androgenic hormonal environment, is more common among adult men and postmenopausal women (364, 365), and

associated with higher cardiovascular disease risk (366). Sex-specific associations between anthropometric measures and brain volumes are inconsistent. As aforementioned, higher BMI is associated with lower GMV in Japanese men (367); and with temporal lobe atrophy in women aged ≥70 years (368). Given that the average age of menopause is 52 years in northern European populations (369), our sample most likely includes women who were going through the menopausal transition. Menopause influences the sex hormone milieu (370), as well as body composition (365), including adipose tissue and consequently, blood adipokine levels.

Differential associations between adiposity measures and brain outcomes may also be dependent on age of measurement and temporality. A changing role of adipose tissue throughout aging and in later life, by age decade, may be a real phenomenon. Our sample was examined on average, during their 7th and 8th decades of life. During this aging period, an inflection point in vascular risk factor trajectories has been observed. These trajectories are described for BMI, blood pressure and blood lipids in association with late-onset sporadic dementias (371-373). While we cannot evaluate life course trajectories in RUN DMC given a 9-year follow-up of adults age 50 years and older, their potential contribution to a changing risk profile, particularly among women, cannot be ignored. Midlife obesity has been associated with reduced GMV and greater dementia risk, whereas later life overweight and obesity has been associated with lower dementia risk (3, 343, 351, 368, 374). Given cross-sectional findings in RUN DMC, we cannot elucidate causality, nor gain insights regarding temporality of these associations.

Lack of associations between higher levels of adiposity measures and CSVD components, may be due to an attenuation of the role of vascular risk factors among those presenting with cerebrovascular disease, similar to observations in dementia epidemiology among those with prodromal dementias such as VCI (371, 375, 376). Given that aging- and dementia-related neurodegenerative events affect homeostatic regulation of energy balance, blood pressure and other aspects of metabolism, temporal paradoxical associations are often observed between vascular risk factors, such as obesity, and symptomatic, clinical outcomes (3, 77, 337, 363).

CSVD is a diagnosis that is evolving over time due to emerging phenotypic characterization and accumulating mechanistic evidence in association with clinical outcomes. CSVD is a group of pathological processes with a heterogeneous etiology and pathogenesis that involve the small arteries, arterioles, venules, and capillaries of the brain. In 2006, the baseline year of the RUN DMC, CSVD was diagnosed on the basis of the presence of any WMH and the presence of any lacunes (377), with accompanying acute symptoms (transient ischemic attacks or lacunar

syndromes), or subacute manifestations (cognitive, motor (gait) and/or mood disturbances) (378). Later recognized markers of CSVD, such as microbleeds, were also rated in RUN DMC. Therefore, our approach of evaluating adiposity measures in association with individual components of CSVD is appropriate, in addition to being more informative for intervention development.

Longitudinal analyses of risk factors for CSVD and its components are challenged since WMH, lacunes and microbleeds change over time in ways that are difficult to characterize. In RUN DMC, it was reported (324) that CSVD progression is nonlinear, accelerating over time, and is a dynamic process, with progression interrupted by reduction in some adults who, on average, show progression. RUN DMC has also reported the disappearance of lacunes and microbleeds over this 9-year follow-up period. In RUN DMC, these unexpected changes tended to occur among those presenting with a worse CSVD phenotype at baseline. Since RUN DMC participants who died during the 9-year follow-up had worse baseline CSVD and volumetric phenotypes compared to those who survived and participated in 2015, and there is worsening of CSVD on average among surviving participants, our longitudinal results are not as likely influenced by these unexpected changes.

Major strengths of our analyses are the following. First, this report is based on 9-year follow-up of men and women presenting with a distinct cerebrovascular phenotype, CSVD, with quantified neurodegenerative outcomes, notably brain volumetry, and thorough clinical and risk factor evaluation. Second, brain imaging data were analyzed by raters who were blind to clinical information, with good inter- and intra-rater agreement. Third, statistical analyses were adjusted for multiple potential confounders. Fourth, while our primary exposure was adiposity, we measured this phenotype in several ways. Using anthropometric (total and central obesity) and metabolic (leptin and adiponectin) markers of adipose tissue, differential associations were observed. This may point to different mechanistic pathways linking adipose tissue biology to neurodegeneration and cerebrovascular events. Fifth, multiple brain imaging outcomes were investigated. This is important for more precise determination of etiologic mechanisms associated with adiposity.

Despite the aforementioned strengths, several limitations should be addressed. First, serum leptin, adiponectin and lipid levels, were not measured at the follow-up examination; blood was only collected at baseline. Second, sociodemographic and health status information was not updated at follow-up in the RUN DMC. Third, nutritional intake and physical activity, known influencers of overweight and obesity, were not measured. Fourth, RUN DMC participants were all diagnosed with CSVD, which makes it difficult to generalize these results to an otherwise 'healthy' older population. It is also known that CSVD brain pathologies exist

without symptomatic burden ('silent' or preclinical CSVD) and are relatively common among elderly. Fifth, the RUN DMC sample is comprised of Dutch adults of northern European ancestry. These results require replication in heterogeneous samples due to global variations in anthropometric and brain characteristics (315, 379). Sixth, due to the small number of adults developing dementia, it could not be evaluated as a clinical endpoint. Finally, no information was available on menopausal status of women.

In conclusion, our analyses demonstrate sex differences in a potential manifestation of the fat-brain axis, and different cross-sectional and longitudinal adiposity-brain associations with aging over a 9-year period in men and women with CSVD. Future research is necessary to elucidate biological mechanisms and fat-brain associations over the life course. Elucidation of both independent and interdependent neuropathological versus vascular mechanisms underpinning the evolving aging brain is imperative.

5

A 10-YEAR FOLLOW-UP OF ADIPOSITY AND DEMENTIA IN SWEDISH ADULTS AGED 70-YEARS AND OLDER

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Abstract

Adiposity measured in mid- or late-life, and estimated using anthropometric measures such as body mass index (BMI) and waist-to-hip ratio (WHR), or metabolic markers such as blood leptin and adiponectin levels, is associated with late-onset dementia risk. However, during later life this association may reverse and aging- and dementia-related processes may differentially affect adiposity measures. We explored associations of concurrent BMI, WHR, and blood leptin and high molecular weight adiponectin levels with dementia occurrence.

924 Swedish community-dwelling elderly without dementia, aged 70 years and older, systematically-sampled by birth day and birth year population-based in the Gothenburg city region of Sweden. The Gothenburg Birth Cohort Studies are designed for evaluating risk and protective factors for dementia. All dementias diagnosed after age 70 for 10 years were identified. Multivariable logistic regression models were used to predict dementia occurrence between 2000-2005, 2005-2010, and 2000-2010 after excluding prevalent baseline (year 2000) dementias. Baseline levels of BMI, WHR, leptin and adiponectin were used.

Within 5 years of baseline, low BMI (<20 kg/m^2) was associated with higher odds of dementia compared to those in the healthy BMI category (≥ 20-24.9 kg/ m^2). Compared to the lowest quartile, leptin levels in the second quartile were associated with lower odds of dementia in women ($P<0.05$).

In late-life, anthropometric and metabolic adiposity measures appear to be differentially associated with dementia risk. While BMI and leptin levels are highly positively correlated, our results show that their association with dementia at age ≥ 70 years, is asynchronous. These data suggest that with aging, the complexity of the adiposity exposure may increase and suggests metabolic dysregulation. Additional studies are needed to better understand this complexity.

Introduction

In later life, lower body mass index (BMI), being underweight by definition, and declining BMI (despite one's BMI level at onset of decline), are associated with greater risk of late-onset dementias (3, 380). In mid- or adult life, higher BMI is prospectively associated with dementia risk (3). This difference in the mid-life *versus* late-life BMI-dementia risk association is often referred to as the 'obesity paradox' and evidence of reverse causality (315).

Anthropometric characteristics are sculpted by amount and distribution of adipose tissue. Changes in this important tissue result from lifetime variations in energy balance, sociocultural background, and epigenetic phenomena, and develop secondarily to central pathophysiological changes in preclinical and clinical dementia (381). Therefore, adiposity indicators like BMI, waist-hip ratio (WHR), and circulating blood levels of adipokines, such as leptin and adiponectin, are potential risk markers for dementia (88, 382-384). Since adipose tissue is the primary source of both of these adipokines, leptin and adiponectin are good biomarkers of this tissue's metabolic activity.

There is limited and ambiguous published research regarding the link between adipokine levels and sporadic, late-onset dementias (360, 385). However, leptin is associated with insulin resistance and brain health (77, 88), and is the adipose tissue hormone (111) most studied in association with brain structure and function. Leptin has numerous effects on brain development (110), and potentially on brain health in cognition and aging. Peripheral leptin enters the central nervous system and interacts with specific areas of the brain such as the hypothalamus and hippocampus (386). In addition to crossing the blood brain barrier (BBB) (387), several studies indicate that leptin is also produced in the brain, for example in the hypothalamus, cortex and cerebellum (388, 389). Leptin has been prospectively associated with dementia over 8 years (111), but not over 24 years, from mid- to late-life (368). Leptin is positively correlated with total and central anthropometric adiposity measures (390, 391).

Adiponectin is a visceral adipose tissue marker and exists as complex multimeric isoforms comprised of high molecular weight (HMW) hexamers and trimers (392). It is an effective insulin sensitizer, and circulating levels are inversely correlated with insulin resistance, metabolic syndrome, adiposity, type 2 diabetes mellitus, and cardiovascular diseases. Adiponectin modulates inflammatory responses, energy expenditure (CNS and periphery), food intake (CNS), and a number of metabolic processes, including glucose regulation and fatty acid catabolism in the periphery. Since BBB transport mechanisms for adiponectin are unclear, blood levels may not indicate potential interactions between adiponectin and the brain (393).

We investigated the association between dementia occurrence and both anthropometric and adipokine indicators of adiposity - BMI, WHR, and blood leptin and adiponectin levels - in a 10-year prospective population-based study of Swedish adults, aged 70 years and older. Moreover, we examined whether these adiposity markers predict dementia occurrence over the short- and/or long-term. This study will further reveal the complex interplay between surrogate markers of adiposity and dementia onset.

Methods

Participants

Participants comprising the Gothenburg Birth Cohort Studies, originate from two epidemiological studies in Gothenburg, Sweden: 1) the Prospective Population Study of Women (PPSW) and 2) the Gerontological and Geriatric Population Studies (H70). Both have been described in detail previously (394-396). Eligible participants were sampled from the Swedish Population Register based on birth date. PPSW participants were born in 1908, 1914, 1918, 1922 and 1930. H70 participants were born in 1930. Adults living in private households and residential care were included. There were 1725 eligible individuals in 2000-2001, and 1018 agreed to participate (response rate 59.0%). Among these, 857 (84.2%) consented to genetic analyses. Due to the nature of PPSW and H70, women (n=700) were 70-92 years and men (n=224) 70 years. Participants included 339 H70 females at age 70, 224 H70 males at age 70, and 361 PPSW females age 70 years and older (Fig. 1). Follow-up examinations were conducted in 2005-06 and 2009-10, and 689 participated in 2005-06 (response rate among survivors, 82.2%), and 518 in 2009-10 (response rate among survivors, 76.7%).

Those who died, or declined participation during follow-up, were traced in records from hospitals and homes for the aged, inpatient and outpatient departments in psychiatric hospitals and clinics, municipal psychiatric outpatient departments in Gothenburg, the hospital-linkage system, and death certificates (397). All participants (or their closest relatives) gave informed consent, which was conducted in accordance to the provisions of the Helsinki Declaration. The study was approved by the Ethics Committee for Medical Research at the University of Gothenburg.

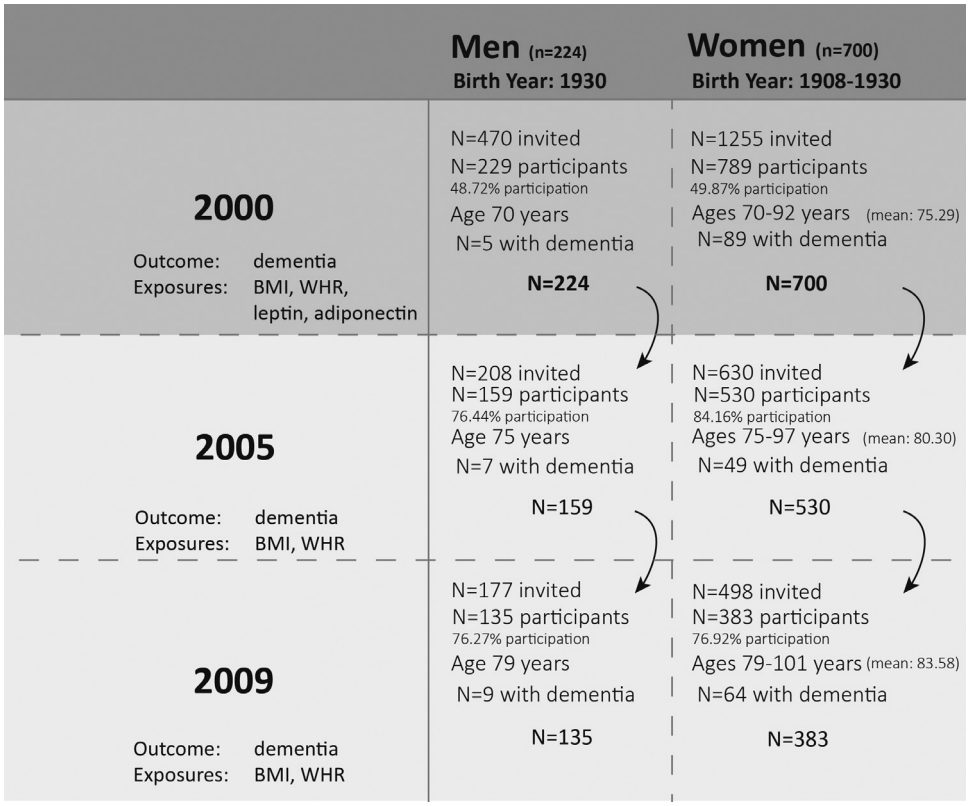

Figure 1. Flowchart of participant characteristics and number at baseline and in follow-up examinations.

Dementia assessment

Dementia diagnoses were made according to the diagnostic and statistical manual of mental disorders 3rd edition revised (DSM-III-R) (334, 398) at examinations in 2000-2001, 2005-2006 and 2009-2010. This dementia diagnosis was made according to components of the Alzheimer's disease assessment scale-cognitive subscale (ADAS-Cog). A close informant interview was conducted using IQCode (399). Symptom domains relevant for making a dementia diagnosis include: memory (short-term, long-term), aphasia (language disturbance), apraxia (inability to carry out motor activities despite intact function), agnosia (failure to identify or recognize objects despite intact sensory function), executive function (planning, organizing, abstract thinking) and personality changes (used in DSM-IIIR criteria for dementia, not in DSM-IV or DSM-V). Participants must have evidenced significant impairment in social or occupational functioning representing significant decline from a previous level of functioning. There was also a global rating of symptom status made by the interviewer (psychiatrist or psychiatric

nurse) and information from close informants related to dementia symptoms. The comprehensive psychiatric rating scale (CPRS) (400), an extensive neuropsychiatric inventory, was used to rate psychiatric symptoms and signs at face-to-face examinations. The mini-mental examination (MMSE) was used as an additional evaluation of global cognitive impairment, but is not considered diagnostic by itself. In addition, the Swedish Hospital Discharge Registry provided diagnostic information for individuals discharged from hospitals since 1978. Medical records were collected from hospitals and outpatient departments in Sweden's public health care system. Incident cases of dementia up to December 31, 2010 were also based on information from the Swedish Hospital Discharge Register (ICD-10: F00.1, F01.8, F01.9, F03.9, G30.9). Thus, dementia diagnoses were obtained for all study participants, since almost all people in Sweden receive health care from the community and have an equal chance of having a medical record. Dementia was diagnosed by neuropsychiatrists at consensus meetings based on all information sources available, e.g., neuropsychiatric examinations, medical records, and close informant interviews.

Anthropometric assessments

Anthropometric measurements were conducted in the morning when participants wore light clothing at each examination (401). Body weight was recorded to the nearest 0.1 kg, and body height was measured to the nearest 0.5 cm. BMI was calculated as kg/m^2. Waist and hip circumferences were measured to the nearest 0.5 cm. Measures of each were conducted until there was agreement within 0.5 cm.

Blood measures

Blood samples were collected after a 12-hour fast, and plasma aliquots stored at −70°C. Regarding baseline adipokine measures, control standards and participant samples were tested in duplicate using high molecular weight (HMW) adiponectin and leptin ELISA assays (Linco Research, Inc, St. Louis, MO 63304) in the Clinical Chemistry Department at the University of Gothenburg. Blood lipids including cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides, were measured at the certified Sahlgrenska Hospital Laboratory.

DNA was extracted from blood samples according to standard procedures. *APOE* (gene map locus 19q13.2) genotyping was performed by mini-sequencing as previously described in detail (402). Genotypes were obtained for the two SNPs (rs7412 and rs429358), which are used to unambiguously define ε2, ε3, and ε4 alleles.

Lifestyle assessments and medical history

Level of education (completing six years compulsory education or less *vs* at least

compulsory education); socioeconomic status (SES, working *vs* middle/upper class); alcohol consumption and cigarette smoking (ever *vs* never use); medication use; and medical history were queried at each examination. Diagnoses of myocardial infarction, stroke, cancer, and diabetes were self-reported as well as determined by clinical examinations (ECG and blood samples), case records, hospital discharge registry, national cancer registry, and national stroke registry. Systolic (SBP) and diastolic blood pressures (DBP) were measured while participants were sitting down and at rest for at least 5 minutes. Depressive symptoms were measured using the Montgomery–Åsberg depression rating scale (MADRS) (403).

Statistical analyses

Means and standard deviations were calculated for all continuous variables, and frequencies and percentages for categorical variables. BMI, WHR, leptin and adiponectin were used in analyses as continuous, and as traditional quartiled categorical variables. We considered baseline adiposity exposures at 2000 and 2005, and percent BMI change from 2000-2005 in association with dementia occurring between 2005 and 2010. Clinically relevant BMI change was categorized as +/- > 5%, and +/- >10%. We created combined anthropometric and adipokine categories as potentially better surrogates of adiposity-associated risk (404, 405). We cross-tabulated leptin quartiles by the four traditional BMI (underweight, <20.00 kg/m^2; healthy, 20.00 - 24.99 kg/m^2; overweight, 25.00 - 29.99 kg/m^2; and obese ≥30.00 kg/m^2) categories. In other words, those with a low leptin and low BMI were compared to those with a high leptin and high BMI, versus either alone. Similarly, leptin quartiles were cross-tabulated by WHR (central obesity: men >0.90 cm, women >0.85).

Multivariable logistic regression analyses were used to calculate odds ratios (OR) for developing dementia over different time periods. First, regression models estimated the odds of dementia over ten years (2000-2010) given adiposity exposures measured at baseline in 2000. Second, we estimated the association between baseline adiposity indicators measured in 2000 among those without dementia and subsequent dementia occurring during two five year periods, 2000-2005 and 2005-2010. To reiterate, prevalent dementia cases at baseline were excluded from all models. In models predicting dementia by adipokines, we adjusted for BMI if the correlation between adipokine and BMI did not exceed $r \geq 0.70$.

Selection of covariates originated from a pool of potentially biologically relevant variables: age; sex; education; socioeconomic status (SES); smoking; alcohol intake; depressive symptoms; diabetes; cancer; heart attack; stroke/transient ischemic attack; hypertension; blood triglyceride, cholesterol, HDL, LDL, and glucose levels; DBP; SBP; and APOEε4 genotype. All potential covariates were included in age-adjusted

logistic regression models predicting dementia over the entire follow-up period. Age, sex, SES, smoking status (ever/never), diabetes (yes/no), alcohol intake (ever/never), depressive symptoms (MADRS score > 12), and presence of at least one APOEε4 allele were significant at P≤0.05 and included in multivariable models. Two-tailed tests were used with a significance level of P≤0.05. SPSS, version 20.0 (IBM Corporation, Armonk, New York, USA), was used to perform data analyses.

Results

Overall, 924 adults (224 men and 700 women) without dementia, participated in the baseline (year 2000) examination. PPSW and H70 were representative of the population base with regard to sex; marital status; income; community rent allowance for those who could not afford housing; rate of inpatient and outpatient care in psychiatric hospitals, clinics, and municipal outpatient departments; and rates of registration with the Temperance Board (national registry for alcohol abuse). Over ten years, 134 participants developed dementia.

The average age of the participants at baseline (n=924) was 74.0 years, and when stratified by sex, the average age of men was 70.0 years and for women, 75.3 years (Table 1). Compared to men, women were more likely to be underweight and had higher plasma levels of leptin and adiponectin. Approximately 25% of the study population had at least one APOEε4 allele (27.7% in men and 24.4% in women).

Multivariable logistic regression models were used to examine the relationship between continuous baseline adiposity indicators and subsequent dementias. No associations were found between BMI, WHR, leptin or adiponectin and dementia occurrence in the entire sample over the ten year follow up (Table 2; data not shown for adipokines).

To better understand the temporality of association between adiposity markers and dementia, we divided the ten year follow-up period into two five year dementia occurrence intervals - 2000-2005 and 2005-2010 (Table 2), as has been done previously (406). When analyzed continuously, a higher baseline BMI lowered the odds of dementia within five years. However, using traditional BMI categories, participants with an 'underweight' BMI (< 20 kg/m^2) had higher odds of developing dementia compared to participants with a 'healthy' BMI (reference category BMI 20.00-24.9 kg/m^2) (Table 2). This finding among underweight may have driven the protective association observed when BMI was modelled as a continuous variable. This association was driven by female participants (Table 2).

Variable	**All** (n=924)	**Men** (n=224)	**Women**(n=700)
Age, years, mean ± SD	74.0 ± 5.3	70.0 ± 0.0	75.3 ± 5.5
Education <8 years, N (%)	544 (58.9)	125 (55.8)	419 (59.9)
Socioeconomic status (SES)			
working class, N (%)	351 (38.0)	57 (25.4)	294 (42.0)
middle or upper class, N (%)	300 (32.5)	100 (44.6)	200 (28.6)
Waist-hip ratio (WHR), mean ± SD	0.87 ± 0.083	0.95 ± 0.06	0.84 ± 0.07
Low WHR: men ≤ 0.90, women ≤ 0.85, N (%)	375 (40.6)	33 (14.7)	342 (48.9)
High WHR: men > 0.90, women > 0.85, N (%)	509 (55.1)	186 (83.0)	323 (46.1)
BMI, kg/m^2, mean ± SD	26.7 ± 4.2	29.9 ± 3.9	26.6 ± 4.3
≤20.00, N (%)	28 (3.0)	4 (1.8)	24 (3.4)
20.01 - 24.99, N (%)	290 (31.4)	66 (29.5)	224 (32.0)
25.00 - 29.99, N (%)	375 (40.6)	108 (48.2)	267 (38.1)
≥30.00, N (%)	180 (19.5)	45 (20.1)	135 (19.3)
Leptin (ng/mL), mean ± SD	31.6 ± 24.0	15.07 ± 10.5	37.28 ± 24.7
Q1 (ng/mL)	≤13.36	≤8.06	≤18.65
Q2 (ng/mL)	13.37 - 25.24	8.07 - 12.04	18.66 - 30.52
Q3 (ng/mL)	25.25 - 44.00	12.05 - 21.07	30.53 - 50.83
Q4 (ng/mL)	>44.00	>21.07	>50.83
Adiponectin (ng/mL), mean ± SD	6.10 ± 4.54	3.52 ± 2.34	6.97 ± 4.77
Q1 (ng/L)	≤3.11	≤1.97	≤3.76
Q2 (ng/L)	3.12 - 4.89	1.98 - 3.01	3.77 - 5.71
Q3 (ng/L)	4.90 - 7.84	3.02 - 4.57	5.72 – 8.90
Q4 (ng/L)	>7.84	>4.57	>8.90
Alcohol consumption			
g per week, mean ± SD	37.5 ± 66.9	72.2 ± 101.9	25.63 ± 44.4
Smoking, ever, N (%)	353 (38.2)	151 (67.4)	202 (28.9)
Serum levels (mmol/L)			
Triglycerides, mean ± SD	1.4 ± 0.7	1.41 ± 0.6	1.46 ± 0.8
Cholesterol, mean ± SD	5.9 ± 1.1	5.5 ± 0.9	6.1 ± 1.0
High density lipoprotein (HDL), mean ± SD	1.6 ± 0.4	1.3 ± 0.4	1.7 ± 0.4
Low density lipoprotein (LDL), mean ± SD	3.7 ± 0.9	3.6 ± 0.9	3.8 ± 0.9
Systolic blood pressure, mean ± SD	157.7 ± 22.5	155.1 ± 23.1	158.2 ± 22.3
Diabetes mellitus, N (%)	111 (12.0)	41 (18.2)	70 (9.9)
Depression,* N (%)	99 (10.7)	15 (6.7)	84 (12.0)
Presence of any Apoe ε4 allele,† N (%)	233 (25.2)	62 (27.7)	171 (24.4)

Table 1. Baseline characteristics of participants without dementia in the Gothenburg Birth Cohort Studies.*Clinically relevant depressive symptom burden, MADRS score > 12. †APOEε4 allele: apolipoprotein epsilon 4 allele.*

In women, we also found an association between leptin levels and odds of dementia within the first five years of follow up (2000-2005, Table 3). No associations were observed in men. Baseline leptin levels in the second quartile (18.66 - 30.52 ng/mL) were associated with lower odds of developing dementia compared to leptin levels in the first quartile (≤18.65 ng/mL, reference). No associations were found between serum levels of HMW adiponectin and dementia occurrence.

Considering only dementias occurring during 2005-2010, there were no associations with baseline BMI, WHR, leptin or adiponectin (Table 2). In addition, BMI change from 2000-2005 was not associated with dementias occurring from 2005-2010 (data not shown). Combinations of anthropometric BMI or WHR risk categories by leptin quartiles did not shed any additional light on these associations (data not shown).

	2000-2010			**2000-2005**			**2005-2010**		
	Total	Men	Women	Total	Men	Women	Total	Men	Women
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Baseline BMI (kg/m²)									
Continuous	0.94 (0.86-1.02)	0.99 (0.85-1.10)	0.92 (0.83-1.01)	0.88 (0.79-0.97)*	0.89 (0.67-1.18)	0.87 (0.78-0.97)**	0.99 (0.89-1.11)	0.99 (0.83-1.21)	0.98 (0.86-1.12)
<20.0	2.94 (0.73-11.88)	NE†	4.59 (0.95-22.22)	4.21 (1.03-17.21)*	NE	5.34 (1.19-23.87)	1.12 (0.13-9.41)	NE	1.32 (0.14-11.45)
20.00 - 24.99 †	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
25.00 - 29.99	0.79 (0.39-1.56)	1.34 (0.34-5.24)	0.67 (0.29-1.50)	0.13 (0.25-1.19)	0.39 (0.04-3.37)	0.54 (0.24-1.27)	0.69 (0.28-1,74)	1.91 (0.33-11.26)	0.45 (0.14-1.43)
≥30.00	0.93 (0.39-2.22)	0.80 (0.13-5.10)	0.92 (0.34-2.51)	0.25 (0.19-1.54)	0.51 (0.04-6.94)	0.51 (0.16-1.66)	1.21 (0.41-3.57)	0.76 (0.06-9.29)	1.29 (0.38-4.40)
Baseline WHR (cm)									
Continuous	1.07 (0.69-1.65)	2.95 (0.92-9.42)	0.87 (0.54-1.39)	0.95 (0.59-1.51)	0.80 (0.17-3.80)	0.96 (0.59-1.57)	1.20 (0.65-2.24)	4.75 (1.03-21.78)	0.81 (0.42-1.55)
Low WHR†	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
High WHR	0.80 (0.42-1.52)	3.13 (0.37-26.52)	0.62 (0.30-1.28)	0.63 (0.32-1.27)	0.54 (0.05-5.45)	0.63 (0.31-1.32)	1.11 (0.47-2.65)	NE	0.77 (0.29-2.06)

Table 2. Odds of developing dementia from 2000-2010, 2000-2005, and 2005-2010 by baseline BMI and WHR among participants without dementia. The Gothenburg Birth Cohort Studies. *Multivariable models included the following covariates: in 2000-2010, age, sex, socio-economic status (SES), ApoEε4 allele, and smoking; in 2000-2005, age, sex, depressive symptoms and SES; in 2005-2010, age, sex and smoking. †Reference category. Multivariable models were assessed for the complete cohort (total), and subsequent for both men and women in the cohort. Low WHR: men ≤ 0.90 cm, women ≤ 0.85; High WHR: men > 0.90 cm, women > 0.85. Number of incident dementias in 2000-2010, N=134 (men N=18 and women N=116); in 2000-2005, N=68 (men N=8 and women N=60); and in 2005-2010, N=66 (men N=10 and women N=56). * NE: not estimable due to small number. *P<0.05; ** P<0.001.*

			2000-2010			2000-2005			2005-2010		
			Total	Men	Women	Total	Men	Women	Total	Men	Women
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Baseline leptin (ng/mL)											
Continuous			0.55 (0.23-1.30)	0.96 (0.18-5.16)	0.48 (0.17-1.31)	0.33 (0.11-0.99)*	0.42 (0.04-4.33)	0.36 (0.1-1.0)*	0.82 (0.23-2.89)	1.76 (0.17-18.70)	0.56 (0.12-2.56)
Quartiles											
	Men	Women									
Q1†	≤8.06	≤18.64	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Q2	8.07-12.04	18.65-30.52	1.15 (0.51-2.63)	0.30 (0.06-1.55)	1.87 (0.69-5.10)	2.62 (0.73-9.42)	1.91 (0.21-23.52)	0.32 (0.10-0.97)*	0.65 (0.22-1.89)	0.35 (0.05-2.65)	0.92 (0.25-3.36)
Q3	12.05-21.07	30.53-50.83	1.17(0.51-2.71)	0.56 (0.11-2.67)	0.63 (0.59-4.52)	3.47 (1.0-12.02)	1.65 (0.12-22.41)	0.82 (0.32-2.04)	0.37 (0.10-1.31)	0.29 (0.03-3.13)	0.35 (0.07-1.73)
Q4	>21.07	>50.83	0.33 (0.07-1.59)	0.44 (0.08-2.57)	0.79 (0.26-2.47)	0.66 (0.11-3.83)	NE	0.45 (0.13-1.13)	0.39 0.08-1.99)	0.69 (0.10-4.58)	0.62 (0.25-2.18)
Baseline adiponectin (ng/mL)											
Continuous			1.14 (0.38-3.40)	0.49 (0.07-3.45)	1.63 (0.42-6.33)	1.63 (0.32-8.31)	1.18 (0.06-25.13)	1.77 (0.26-12.22)	0.89 (0.22-3.54)	0.30 (0.03-3.29)	1.53 (0.25-9.30)
Quartiles											
	Men	Women									
Q1†	≤1.97	≤3.76	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Q2	1.98-3.01	3.78-5.71	0.39 (0.15-1.51)	0.19 (0.02-1.74)	0.49 (0.16-1.48)	0.43 (0.10-1.82)	1.22 (0.07-22.11)	0.29 (0.05-1.65)	0.36 (0.10-1.24)	NE	0.67 (0.16-2.73)
Q3	3.02-4.57	5.73-8.90	0.74 (0.31-1.07)	0.89 (0.21-3.83)	0.62 (0.22-1.77)	1.06 (0.33-3.43)	2.52 (0.19-34.24)	0.81 (0.21-3.13)	0.53 (0.18-1.61)	0.53 (0.09-2.91)	0.44 (0.09-2.03)
Q4	>4.57	>8.90	0.93 (0.42-2.08)	0.38 (0.06-2.27)	1.19 (0.47-3.03)	1.19 (0.38-3.77)	1.19 (0.07-21.63)	1.08 (0.31-3.76)	0.79 (0.28-2.21)	0.22 (0.02-2.23)	1.29 (0.36-4.56)

Table 3. Odds of developing dementia from 2000-2010, 2000-2005, and 2005-2010 by baseline leptin and adiponectin among participants without dementia. The Gothenburg Birth Cohort Studies. *Multivariable models include in 2000-2010, age, sex, social economic status (SES), ApoEε4 allele, and smoking; in 2000-2005, age, sex, depressive symptoms and SES; in 2005-2010, age, sex and smoking. †Reference category. Multivariable models were assessed for the complete cohort (total), and subsequent for men and women. In adiponectin analyses BMI was included as covariate. NE: not estimable due to small number. Number of incident dementias in: 2000-2010, N=134 (men N=18 and women N=116), 2000-2005 N=68 (men N=8 and women N=60).*

Discussion

In an elderly Swedish sample of women and men, we observed that a low BMI (<20 kg/m2) was associated with over a 5-fold higher odds of developing dementia within five years in women, aged 70 years and older. Given published literature on mid-life obesity and later onset dementia, this may be evidence of an obesity paradox and/or reverse causality. However, without mid-life measures, we cannot be sure. In addition, the odds of developing dementia were 70% lower in women aged 70 years and older who had intermediate serum leptin levels based on analysis of leptin quartiles. Thus, despite a robust correlation of r =0.71 between BMI and leptin in this sample, there is a suggestion that these adiposity indicators were differentially associated with development of dementia within 5 years of measurement in elderly women.

Other studies in middle-aged and elderly women have suggested contradictory associations between anthropometric versus leptin levels and cognition or dementia (360, 407, 408).

Our analyses support a first-ever published report from the original Gothenburg Birth Cohort Study (H70) among those born 1901/02 (406). Similar to the 2003 report, there was no association between higher BMI and dementia observed during the first 10 years of follow-up. In contrast to the 2003 report, the current analysis includes primarily those born 1930. Thus, with further follow-up, we will be able to observe a 30 year cohort comparison of the BMI-dementia association. In addition, we also chose to focus on all dementias, due to controversies and changes in the field regarding diagnoses of dementia subtypes without the use of neuroimaging or cerebrospinal fluid biomarkers (409).

Our study has many advantages. First, the population sample is part of a long-standing series of rich longitudinal birth cohort studies in Sweden beginning in 1968 (PPSW) and 1971 (the first H70 cohort) (394, 395), with consistent measures over time among unique birth cohorts. Second, there is long follow-up of a large sample of rigorously studied elderly, age 70 years and older, and adjustment for multiple potential confounders. Third, we measured blood levels of adipokines reflecting the metabolic activity of adipose tissue. Finally, we used the study design to our advantage, to estimate temporal (shorter versus longer term) associations of adiposity indicators with dementia. Given the important role of temporality in understanding risk and protective factor-dementia associations, we see this as a definite strength of these studies.

As with any study design, there are limitations. First, while data from the Gothenburg Birth Cohort Studies have been published using this merged sample examined with identical methods by the same research group at the same time (410, 411), the study is unbalanced regarding sex and age. All men were aged 70 years at baseline, whereas women were aged 70 years and older. Thus, our observations regarding sex differences should be interpreted cautiously. Second, a small number of male participants reduced the power for analyzing associations in men given that dementia onset was monitored during a period of relatively low risk, notably the 8th decade of life (406). Third, leptin and adiponectin measures were only measured at baseline, thus repeated measures and change in these important hormones over time cannot be considered. Fourth, other nutritional influencers of BMI and WHR, including estimates of energy intake and expenditure, are unavailable. Fifth, our leptin findings are rather conservative. While leptin has strong mechanistic actions in the brain, the use of this marker in the periphery as a reflection of the fat-brain axis remains unclear, especially at a life stage when there are ageing-related body

composition changes. In addition, replication of our observations regarding leptin is essential. Finally, some may deem four and five year examination intervals to characterize date of dementia onset as unacceptable. However, given the insidious and often slowly progressive course of dementia onset and a population-based sample, we deem it highly acceptable for these types of analyses. Calculating odds ratios (OR), given this nuance, closely approximates the risk. We have shown this in a previous publication on adiposity measures and dementia (406).

While we found that the odds of developing dementia are higher in elderly women with low BMI within five years, this is not a new finding. The association between BMI and dementia appears to change over the life course. Some studies show that adults with a high midlife BMI or central obesity have a higher risk of late-life dementia (3). Others have reported that weight loss or BMI decline is associated with dementia risk in elderly (71, 73). Our data did not suggest associations of baseline BMI and dementias occurring between 2000-2010 nor dementias occurring between 2005 and 2010. Nor was BMI change from 2000-2005 related to dementias occurring between 2005-2010. This suggests temporal issues regarding the BMI-dementia association in this sample, however we cannot evaluate this historically since we do not have measures of BMI prior to 70 years. Thus, a lower BMI may be a preclinical marker for underlying dementia development within five years of a clinical diagnosis in women aged 70 years and older. These results should be interpreted with caution due to a small number of women in the lowest BMI category. The association of lower baseline BMI with dementia may result from a lengthier period of weight loss accompanying prodromal dementia prior to age 70 years, supporting BMI as a possible preclinical marker of dementia.

That low BMI may be a preclinical marker for dementia, is suggested by neuropathophysiological changes accompanying dementia. These changes can induce changes in body weight and adipose storage. For example, brain areas that control weight (i.e. mesial temporal cortex) are affected during the preclinical dementia phase; and adipose tissue loss may result from preclinical apathy, reduced olfactory function, difficulty with eating (e.g., aphasia), inadequate nutrition or prescribed medicines targeting dementia related symptoms e.g. depression or cognitive impairments (337).

Furthermore, we found that the odds of developing dementia in elderly women were lower among those with intermediate blood leptin levels. Postmenopausal women may have a greater risk of developing dementia than men, perhaps due to changes in leptin and brain function due to a fall in endogenous estrogen levels following menopause (412). Estrogen and leptin have neuroprotective effects on cognition by regulating neurogenesis, hippocampal synaptic plasticity, and axonal

growth (77). However, the roles of adipokines are diverse, and often enigmatic. The adiposity-dementia association, remains to be elucidated, as this is by no means clear.

As higher levels of adiposity, commonly measured as anthropometric overweight and obesity, are associated with higher risk for cardiovascular disease (CVD) (85, 87), and CVD is a risk factor for dementia (67), we explored CVD variables as potential covariates. None were significantly associated with dementia in age-adjusted models. This null observation associating dementia with CVD risk in late-life concurs with other published reports (65). In addition, adiponectin, known to be associated with CVD, was not associated with dementia.

The underlying neurodegenerative and vascular pathologies observed in dementia, and subsequent impairment of key fat-brain and gut-brain feedback loops (413), may be at the root of conflicting observations; and seemingly intertwined adiposity factors may be differentially associated with dementia when measured within five years. Our findings are supported by studies reporting that higher levels of leptin are prospectively associated with lower odds of dementia or mild-cognitive impairment in elderly (360, 414, 415). Metabolic dysregulation may accompany or promote late-life alternations in leptin levels and dementias (416). Atrophy of the posterior hypothalamus affects appetite and feeding behavior via disintegration of network connections and effects on hormone synthesis (416, 417). In addition, higher levels of leptin are observed to be neuroprotective (418).

Conclusion

Two highly correlated adiposity variables measured in late life do not associate similarly with late-onset dementia in women. Our analyses show a higher odds of dementia with a low BMI, and a lower odds of dementia with intermediate leptin levels when dementia occurs within 5 years of the adiposity measurement among women aged 70 years and older. Lower BMI and higher leptin may potentially characterize aspects of metabolic dysregulation associated with presence versus absence of prodromal dementia. However, both associations suggest a similar adiposity phenomenon, whether cast as lower BMI being risky or higher leptin levels being protective.

6

GENERAL DISCUSSION AND CONCLUDING REMARKS

General discussion

In 2015, nearly 40% of the adult population in the United States was obese, and the obesity epidemic is still rising (419). Obesity is characterized by accumulation of white adipose tissue (WAT; adiposity), which eventually may have adverse effects on human health and cognition. Accumulation of WAT is potentially modifiable, therefore it is important to identify and better understand affected mechanisms in the adiposity-brain-axis. Eventually, knowledge on the adiposity-brain-axis may facilitate interventions to reduce the impact on public health. Therefore, with the research performed in this thesis we aimed to reveal mechanisms underlying the adiposity-brain-axis, in particular the involvement of cerebrovasculature, adipokines and aging, and the effects of fatty acids in these mechanisms in both mice and men. In this General discussion, it will be considered to what extend the results of the experiments described in the previous chapters may elucidate the adiposity-brain-axis.

Mouse models

Transgenic mice models are commonly used in preclinical obesity research, because in a relatively short period of time diet-induced-adiposity can be provoked, and/or the susceptibility to develop co-morbidities like cardiovascular disorders. In our preclinical studies (**Chapter 2 and 3**), we examined the ApoE3*Leiden and low-density lipoprotein receptor knock-out Leiden (LDLr-/-) mouse models. Both mouse models are susceptible for accumulation of WAT, and the development of cardiovascular disorders like atherosclerosis and hypertension. We elucidated cerebral blood flow (CBF), structural brain changes and functional connectivity in these mouse models using high resolution (11.7 Tesla) magnetic resonance imaging (MRI)-scanner. Thereby, we were, to our knowledge, one of the first examining these parameters at this high resolution in context of rodent obesity. Nevertheless, there are some limitations concerning mouse models. For instance, anesthesia is a limitation within MRI research in rodent models. Anesthesia is stressful, induces hyperglycemia, and some types of anesthesia are stored within WAT (420).

Secondly, metabolic phenotypes vary between mouse strains, and even vary between subpopulations of the same strain. For instance, C57BL/6J (The Jackson Laboratory) mice are predisposed to the obese and diabetic phenotypes, and show impaired insulin secretion compared to C57BL/6N (NIH) mice (421). Moreover, transgenic mouse models are characterized by pathological features. For instance, the LDLr-/- mouse model is susceptible to develop adiposity, hyperlipidemia, and atherosclerosis (**Chapter 3**). In detail, the loss of hepatic low-density lipoprotein receptor (LDLr) expression can increase circulating lipoproteins providing

increased triglyceride substrate for uptake by adipocytes (422). Alternatively, loss of the LDLr may have indirect effects on reducing lipolysis in adipocytes or altering thermogenesis, which would also increase lipid storage in WAT (422). The exact biological mechanisms involving lipid intake and accumulation in specific adipose depots is still under debate in the LDLr-/- mouse model, which might complicate the comparison of findings in this mouse model compared to other mouse models. Therefore, it is important to recognize the details of genetic background when interpreting experimental results, and the affected biological mechanisms involved in transgenic mouse models.

Thirdly, it remains challenging to compare mice to humans, particularly aging effects as for instance mice exert a maturation phase of 12 weeks which includes three weeks of weaning. Thus compared with humans, mice have an accelerated maturation rate during childhood because they develop rapidly during infancy (150 times faster than humans), and generally, at 3–7 months are considered mature adults, equivalent to 20–30 years in human (421, 423). These limitations of mouse models must be taken into account when designing an experiment. Some possible directions to overcome these limitations could be elucidating the exact biological processes affected in transgenic mouse models. And last, the use of a standardized method such as proposed by the ARRIVE guidelines (286). Nevertheless, animal models still have the potential and are indispensable for examining biological mechanisms in anatomical, metabolic, and neurochemical detail.

Cerebral circulation

The influence of adiposity on the vasculature may play a key role in the development of cognitive impairment, and dementia (424, 425). In detail, adiposity can induce atherosclerosis in large cerebral arteries, and more importantly it can impair the cerebral microcirculation (425). Alterations in cerebral circulation can induce a decreased cerebral blood flow (CBF; hypoperfusion) or cerebral small vessel disease (CSVD) (318, 319, 339). CSVD can be diagnosed by markers like white matter hyperintensities (WMH), lacunes and microbleeds using MRI imaging. Biological mechanisms involved are inflammation, loss of pericyte coverage on cerebral microvessels, blood brain barrier disruption, altered hippocampal angiogenic gene expression (282, 317). Alterations within the cerebral circulation can contribute to cognitive impairment as CBF is essential for normal neuronal function (319). Obese individuals often have an increased systolic blood pressure, and reduced CBF (18, 426). In **chapter 3** we reinforce these finding in obese mice. Nevertheless, in a model for mild obesity we found reverse effects in CBF (**Chapter 2**). Plausibly, the blood vessel walls in the relatively mild obese ApoE3*Leiden mouse model were not severely affected or atherosclerotic, and may still be able to compensate the

HFD induced changes. Moreover, we examined adiposity related to brain volumes in **chapter 4**, where we reported that body mass index (BMI), waist circumference (WC), and leptin were negatively associated with total gray matter volume (GMV). Moreover, we found that leptin was negatively associated with severe white matter hyperintensities (WMH) and presence of lacunes, which indicated a protective effect of leptin in relation to CSVD outcomes. Whereas others reported that adiposity indicated by the amount of visceral adipose tissue, is positively associated with CSVD indicated by microbleeds, lacunes, and WMH (318, 339). Possibly, anthropometric measurements like BMI and WC may not be accurate enough to indicate specifically visceral adipose tissue. In addition, CSVD progression seems to be nonlinear, accelerating over time, and seems to be a highly dynamic process (324), which might complicate the over time analyses of the association between adiposity and CSVD markers. Moreover, in humans we used 1.5 Tesla MRI images to indicate CSVD markers. High resolution images, used in our preclinical research (11.7 Tesla), might provide the opportunity to precisely examine microstructural changes within the cerebral circulation. Thus, in rodent models we reinforce the adverse effects of obesity within cerebrovascular pathology. However, in humans more research is necessary to elucidate the effect of obesity on cerebrovascular pathology, in specific brain regions and its progression.

White adipose tissue (WAT)

Adiposity seems to be a key inducer of adverse effects on health, and cause of dysfunction of numerous types of tissues including the brain (112, 117, 321). WAT is a large endocrine organ, secreting hundreds of adipokines, and particularly the endocrine activity of WAT can be deregulated in adiposity (77). Adipokines contribute to the regulation of appetite and satiety, fat distribution, insulin secretion and sensitivity, energy expenditure, endothelial function, inflammation, and blood pressure (427). Therefore, alterations in adipokine secretion may link adiposity to its inflammatory, metabolic, and vascular comorbidities (427). Functional characterization, in particular of the more recently identified adipokines like vaspin, apelin and resistin, is a major task in future adipokine research.

In mouse models, we found that leptin is not increased in mildly obese mice, whereas leptin levels were significantly increased in the obese mice (428)(**Chapter 2 and 3**). In **Chapter 4 and 5**, we reported that obese individuals have increased leptin levels, and women exert a higher level than men. These findings are in coherence with findings in literature (77, 389). Moreover, we reported asynchronous results for leptin in **chapter 4 and 5**, for instance leptin was negatively associated with GMV in men, whereas it was suggested to be protective in relation to CSVD outcomes in men, and dementia in women. These findings might indicate that leptin's effects in

the adiposity-brain-axis are diverse, change with aging and are influenced by sex. Notably, leptin levels are mostly determined in blood plasma, which provides an accurate indication of metabolic activity. However, leptin signaling within the brain depends on transport of leptin across the blood brain barrier, and availability of its receptors in specific brain regions (77). Therefore, further research is necessary to elucidate the exact function of leptin in specific brain regions, aging and sex.

Adiponectin is a protein hormone, most well-described for modulating inflammatory responses, energy expenditure, energy intake and a number of metabolic processes, including glucose regulation and fatty acid catabolism in the periphery (136-138, 140, 429). In the periphery, adiponectin is released from adipose tissue into the blood circulation as full-length trimers, hexamers, high molecular weight, multimers, and a globular fraction called globular adiponectin (430, 431). In humans, plasma adiponectin levels are, in contrast to most adipokines, inversely correlated to an increase in WAT, as well as to surrogate measures such as percentage body fat, waist-to-hip ratio and BMI (432, 433). In **chapter 4** we confirm these findings, nevertheless we found no association between adiponectin and brain volumes, CSVD markers or dementia. In addition, leptin and adiponectin exert insulin-sensitizing effects, they are considered as important regulators of β-cell mass and survival (77, 427). Therefore, both adipokines might influence the development insulin resistance, and eventually type 2 diabetes mellitus. The mouse studies described in **chapter 2 and 3** provide blood glucose and plasma insulin levels, however both studies are limited in examining glucose homeostasis. Following studies should investigate the functionality of α- and β-cells, glucagon, or assess a glucose tolerance test.

Aging

During aging the associations clarifying the adiposity-brain-axis can be rather ambiguous. In detail, midlife obesity is associated with reduced GMV and greater dementia risk, possibly via impairment of cerebral circulation and/or neuroinflammation (3, 343, 351, 368, 374). High adiposity might contribute to neurodegenerative and cerebrovascular changes through vascular and metabolic pathways (434). However, others reported that in humans that midlife overweight and obesity were not associated with infarct-like brain lesions, cerebral microbleeds, or dementia compared with normal weight after a mean follow-up of 26.2 years (435). In **chapter 4**, we found that BMI, waist circumference (WC) and leptin are negatively associated with GMV in adult men (> 50.0 years). Nevertheless, if obesity is included in forecast models prevalence of dementia is estimated to be 9% for the United States, 14 % in Australia, and 19% higher in China, in 2050 (436, 437). In mid-adult obese rodents, a butyrate supplementation served as preventative for

obesity and obesity related co-morbidities (**Chapter 3**). We indicated in this rodent study (**Chapter 3**) that particularly in midlife inflammatory and cerebrovascular mechanisms can be influenced by for instance a butyrate intervention. These findings might indicate that midlife obesity can be a susceptible life period in which a dietary intervention can beneficially effect obesity and its co-morbidities.
Additionally, examining aging in the adiposity-brain-axis is complicated by sex hormones, which induce sex-specific features in WAT storage. For instance, importantly, women differ with respect to distribution of WAT, men tend to accrue more WAT within the visceral adipose depots, which has been highly correlated to increased cardiovascular risk (438). Women on the other hand, accrue more WAT in the subcutaneous depots prior to menopause, a feature associated with protection from the negative consequences associated with obesity and the metabolic syndrome (438). In detail, after menopause, deposition of adipose tissue shifts to favor the visceral adipose depot. This shift is accompanied by a parallel increase in metabolic risk reminiscent to that seen in men (438). In this thesis we found sex differences in associations between adiposity and brain volumes mainly in adult men (> 50.0 years), and between adiposity and dementia particularly in older women (>70.0 years) (**Chapter 4 and 5**). Postmenopausal women may have a greater risk of developing dementia than men, perhaps due to changes in leptin and brain function due to a fall in endogenous estrogen levels following menopause (412). Estrogen seems to protect against adiposity, and it can influence the function of adipokines like leptin and adiponectin (439). Moreover, estrogen and leptin can have neuroprotective effects on cognition by regulating neurogenesis, hippocampal synaptic plasticity, and axonal growth (77). Future studies should elucidate the role of sex-hormones in an aging adiposity-brain-axis.

Weight loss in late life is strongly associated with dementia risk. Underweight in elderly is strongly associated with increased dementia risk within a 5 year follow up (315, 440) (**Chapter 5**), which might be associated with the presence versus absence of prodromal dementia. The biological mechanisms involved in late life weight changes and the risk of dementia are not fully understood but might be associated with pathophysiological changes in brain areas regulating energy intake, behavioral disturbances, and medication (71, 337, 381). Moreover, we found that leptin can be differently associated with dementia odds than BMI in elderly (**Chapter 5**). This might indicate that the role of leptin changes during aging. Our findings are supported by studies reporting that higher levels of leptin are prospectively associated with lower odds of dementia or mild-cognitive impairment in elderly (360, 414, 415). Moreover, plasma leptin levels have been positively correlated with GMV in various brain regions including the hippocampus, and high leptin levels might protect against age-related cognitive decline in an elderly population study

(355). A study of *Bonda et al.* revealed that leptin signaling is deregulated in the degenerating hippocampal neurons in patients with Alzheimer's disease (AD), which may indicate neuronal leptin resistance in AD (356). Metabolic dysregulation may accompany or promote late-life alternations in leptin levels and dementia (416). Thereby, leptin might play a pivot role in an aging adiposity-brain-axis, and possibly by affecting satiety, glucose homeostasis, inflammatory processes, vascular function, and memory formation (112, 117, 321, 357-360). A limitation in human studies is that the interpretation and comparing epidemiological findings is rather complicated as a study population of people born in 1930 exert different anthropometric features as individuals born in 1990, plausibly due to changes dietary habits, socioeconomic environment, and scientific and technological progress.

Thus, obesity in midlife most likely forecasts a higher dementia risk in late life, although in elderly weight loss might moreover indicate presence of prodromal dementia. Future studies could elucidate the progression of structural brain changes affected by adiposity in aging, by plotting for example GMV against an obese and lean population over a life period.

Diets

High fat diets (HFDs) are regularly used to elicit changes in body composition like provoking adiposity, inflammation, non-alcoholic fatty liver disease (NALFD), high level of circulating triglycerides and cholesterol, and cognitive deficits (441, 442). We confirm these effects of a HFD in **chapter 2 and 3**. The duration of HFD feeding is an important factor to consider concerning aim of the study and mouse model. As for instance, in a LDLr-/- mouse model, overweight develops after 6-10 weeks on a 24% w/w HFD, and the first structural brain changes are observed after about 12 weeks on HFD and it requires about 24-32 weeks to develop cognitive impairment. Male mice are generally more sensitive to develop obesity on HFD than female mice. In addition, genetic variation and modification in mouse models can affect metabolic function, and therefore the effects of HFD exposure may differ between mouse models. In most studies on diet-induced obesity, mice are fed on a HFD for 8–15 weeks (421).

In severe obese individuals the standard lifestyle recommendations fail, or is not compatible with modern life routines. Therefore, alternative strategies for intervention are urgently required. Preventive nutritional strategies during critical periods in life seem promising to reduce the risk of obesity development. For instance, omega-3 fatty acids are able to prevent body weight gain, hepatic lipid accumulation by inducing hepatic fatty acid oxidation (421, 443, 444). We revealed that the intake of LCPUFAs, ARA and DHA, in early life can protect against

HFD-induced-obesity in late life (**Chapter 2**). In children daily consumption of DHA and eicosapentaenoic acid (EPA) improved anthropometric and lipid parameters (27). These findings confirm that ARA and DHA dietary intake in early life strengthens the ability to cope with an obesogenic diet. In **chapter 3** we found that butyrate, a short chain fatty acid (SCFA), restores HFD-induced obesity on metabolic, cerebrovasculature and cognitive function. Possible mechanisms by which ARA, DHA and butyrate can protect or restore HFD-induced obesity and co-morbidities is still incomplete. Although, it has been reported that ARA and DHA intake results in variations in adipocyte differentiation, whereby storage of WAT can be affected (262). Moreover, DHA and ARA are the two most abundant LCPUFAs in the brain, and are essential to support optimal brain and visual system development (204, 205). Butyrate has anti-inflammatory properties, contributes to fatty acid oxidation, and can induce a switch from lipid synthesis to lipid utilization (298, 308). In addition, butyrate most likely affects cognitive function via enhancing histone acetylation by inhibiting histone deacetylase (283). Dynamic changes in histone acetylation have been linked to the genetic programming required for memory formation (283). Additionally, adiposity can be affected by the gut microbiome through energy harvesting and lipid storage by the bacteria. Moreover, butyrate is a product of bacterial fermentation of mainly indigestible plant polysaccharides and resistant starch in the colon, and obese individuals have a decreased level of butyrate producing microbiota (23). Furthermore, analysis of the gut microbiome revealed pronounced changes in composition upon HFD feeding (**Chapter 3**). These HFD induced changes were very consistent with observations made by others (312). In line with observations made in obese humans which exhibit decreased levels of butyrate-producing bacteria compared to lean individuals (24), we found HFD induced in *Clostridium XIVa* abundancy (**Chapter 3**). Moreover, the pronounced difference between the microbiome of mice fed a control diet or fed a HFD made it difficult to discriminate the specific effects of butyrate (**Chapter 3**). However, in literature it was reported that both butyrate and omega-3 fatty acids modify microbiome composition, and the expression of anti-inflammatory compounds (445-447). Thereby, butyrate and DHA are able to affect adiposity by modifying microbiome composition. Thus, fatty acids like DHA and butyrate, exert a moreover protective effect in adiposity and its co-morbidities. Nevertheless, it has to be taken into account that dietary interventions might provoke slightly altered effects in other mouse models as their metabolic function may differ due to genetic modification.

In humans, LCPUFAs like ARA and DHA are added in milk formula's and they are beneficially affecting the development of infants (448). In addition, in adults LCPUFAs improve vascular endothelial function and can reduce blood pressure,

circulating lipids, and inflammatory processes (449, 450). We indicated that SCFAs, like butyrate, may serve as potential preventative for obesity and obesity related diseases (**Chapter 3**). However, up till now it is rather difficult for obese individuals to obtain a relatively high concentration of butyrate within their blood plasma. Some adversities to overcome are for example the distinctive odor of butyrate, and relatively low uptake of butyrate in the blood circulation when consuming fiber rich diets (451). Nevertheless, *Van der Beek et al.* implied that rectal butyrate administration was safe at a dose of 100 mmol sodium butyrate/L, and that it led to higher portal butyrate concentrations compared with placebo (452). Future research could address the effectiveness of a lower concentration of butyrate and/or capsules that release butyrate within the intestine.

Concluding remarks of this thesis research

Overall, in this thesis we clearly demonstrate a link between adiposity and brain function. Animal studies have the potential to examine biological mechanisms and dietary interventions within the adiposity-brain-axis in great detail. Moreover, with this thesis we have furthered the understanding of biological mechanisms by which adiposity can affect brain function, e.g. via adipokines like leptin or affecting cerebral circulation. However, it seems to be a highly interactive and multi-factorial process, which is clearly altered by sex and during aging. Therefore, future research should account for age and sex-hormones while examining the adiposity-brain-axis. Moreover, we underline the role of diets in health. Firstly, intake of LCPUFAs (ARA and DHA) in early life prevents WAT accumulation, improves gut microbiome and preserves brain function. Secondly, a butyrate intervention, and plausible the intake of indigestible fibers (butyrate is a product of bacterial fermentation of indigestible fibers), can counteract HFD-induced adverse health effect in midlife in adiposity, gut microbiome, cerebrovasculature, liver and brain function. Thereby, balanced diets containing undigested fibers and LCPUFAs have a crucial role in attenuating the genesis of adiposity and it co-morbidities throughout lifespan.

7

APPENDICES

Summary

Worldwide, obesity has reached epidemic proportions, and each year obesity or obesity-related-diseases lead to the death of 2.8 million adults. Obesity has an undefined aetiology, but is generally caused by imbalance of energy intake versus energy output. This imbalance results in excessive, mainly white adipose tissue (WAT) accumulation, adiposity, which eventually may have an adverse effect on human health, such as cardiovascular disease, type 2 diabetes mellitus and cognitive impairment. The aim of the research presented in this thesis was to elucidate the adiposity-brain-axis involving dietary fatty acids (arachidonic acid (ARA), docosahexaenoic acid (DHA) and butyrate), (cerebro)vasculature, adipokines, and the effect of aging on these processes.

Chapter 1 provides the background information for this thesis research, including the role of diets in the progression, and contrarily the prevention, of adiposity. Moreover, we discuss two pathways via which adiposity might affect brain structure, function and cognition with emphasis on the (cerebro)vascular pathway and adipokines. In short, diets with a high content of saturated fat (HFD) or high fat and high carbohydrates (HFHC), are considered to be important contributing factors of obesity, whereas dietary interventions containing adequate levels of LCPUFAs, butyrate or even fiber rich diets, could contribute to the decline of adiposity. Moreover, we hypothesize that adiposity can induce (cerebro)vascular changes such as arterial stiffness, inflammation and hypoperfusion, which can subsequently disrupt brain structure and function, and increase the risk for cognitive impairment and dementia. Lastly, we speculate that adipokine release may be dysregulated in adiposity, possibly because the adipose tissue may be 'diseased'. Since neurodegenerative and vascular processes observed in dementia affect several brain regions and nuclei, the action of adipokines might be altered during neurodegenerative and vascular events, and may even feedback to contribute to neurodegeneration.

In the first experimental work, described in **chapter 2**, we examined the effect of early life dietary long chain polyunsaturated fatty acids (LCPUFAs, ARA and DHA) intake in mild-obese transgenic ApoE3*Leiden mice. A broad and multidisciplinary experimental approach was used to examine energy metabolism, brain structure, cerebral circulation, and cognition. We found that a HFD diet causes adverse structural brain and metabolic adaptations in mildly obese mice, like a decrease in functional connectivity and gray matter integrity, and an increase in cholesterol and triglycerides levels. Importantly, most of these alterations due to HFD can be averted by dietary intake of ARA and DHA for 8 weeks early in life, which supports metabolic flexibility and cerebral integrity later in life.

In **chapter 3** a severe mouse model of obesity, low-density lipoprotein receptor knock-out Leiden (LDLr-/-) mouse, have been examined for the development of HFD-induced-obesity, inflammation within WAT, increased fat storage within the liver, increased systolic blood pressure, and cerebral hypoperfusion (**Chapter 3**). Subsequently, we aimed to examine the preventive abilities of short chain fatty acid, butyrate in obese LDLr-/- male mice. We found that butyrate restored HFD-induced-obesity parameters towards normal levels in two months in mid-adult obese mice, for instance it restored body weight, neuroinflammation, liver steatosis, cerebral blood flow and functional connectivity. Additionally, we found that a HFD can lower the variance the gut microbiome, and thereby a HFD can affect adiposity through energy harvesting and lipid storage by the bacteria. Moreover, butyrate could modify microbiome composition, nevertheless the pronounced effects of HFD made it difficult to discriminate the specific effects of butyrate. Furthermore, we suggest that butyrate may have potential in prevention and treatment of midlife obesity.

In **chapter 4 and 5** we aimed to analyze associations concerning the adiposity-brain-axis in humans. In the RUN DMC study (**Chapter 4**), we found that adiposity indicators, body mass index (BMI), waist circumference (WC) and leptin are negatively associated with gray matter volume (GMV) in men diagnosed with cerebral small vessel disease (CSVD) at baseline. BMI and WC were also inversely associated with hippocampal volume in men. At follow-up, 9 years later, cross-sectional analyses showed inverse associations between BMI and GMV; and WC and hippocampal volume, particularly in men. In relation to CSVD outcomes, the metabolic adiposity indicator, leptin, was protective in men. In particular, the highest quartile of leptin (compared to the first quartile) was inversely associated with severe white matter hyperintensities, the presence of any lacunes and the presence of at least one component of CSVD. These findings indicate that adiposity measures, both anthropometric and metabolic were differentially associated with brain volumes and CSVD, and by sex. These findings suggest an Alzheimer-vascular spectrum associated with adiposity, a known marker of both neurodegeneration and vascular risk, that both influences and is influenced by brain pathologies associated with dementia.

In **chapter 5**, we examined in older Swedish adults individuals participating in the Gothenburg studies. These Gothenburg studies provided us a 10-year prospective population-based study of 70 years and older inhabitants of Gothenburg, Sweden. Subsequently, we analyzed if anthropometric parameters as BMI and waist-hip ratio, and serum adipokines levels like adiponectin and leptin could predict dementia risk in older Swedish adults over a 5 and 10 year period. In addition, we examined whether these associations are different between older men and women.

We found that in late-life, anthropometric and metabolic adiposity measures appear to be differentially associated with dementia risk in women aged 70 years and older (Gothenburg). In detail, while BMI and leptin levels are highly positively correlated, our results show that their association with dementia at age ≥ 70 years, is asynchronous. Leptin is associated with decreased dementia risk, whereas low BMI (<20 kg/m^2) predicted an increased dementia risk over a 5 year period.

In **chapter 6**, we provide an extensive discussion of the research findings summarized above, and furthermore we propose follow-up studies are needed to address the questions that have arisen from our work. The most important conclusions of this thesis research are: 1) A clear link between adiposity and brain function. 2) The potential of animal studies to examine biological mechanisms and dietary interventions within the adiposity-brain-axis in great detail. 3) Intake of LCPUFAs in early life prevents WAT accumulation, and preserves brain function. 4) Butyrate intervention and plausible the intake of indigestible fibers (butyrate is a product of bacterial fermentation of indigestible fibers), can counteract HFD-induced adverse health effect in midlife in adiposity, gut microbiome, cerebrovasculature, liver and brain function. 5) The adiposity-brain-axis is a highly interactive and multi-factorial process, which is clearly altered by sex and during aging. Overall, we think that the research described in this thesis has furthered the understanding of biological mechanisms by which adiposity can affect brain function. Thereby, balanced diets containing undigested fibers and LCPUFAs have a crucial role in attenuating the genesis of adiposity and it co-morbidities throughout lifespan.

Nederlandse Samenvatting

Obesitas is uitgegroeid tot een wereldwijde epidemie, en elk jaar resulteren obesitas of obesitas-gerelateerde-ziekten in de dood van 2.8 miljoen volwassenen. Obesitas heeft onbepaalde etiologie, maar wordt over het algemeen veroorzaakt door een disbalans tussen energie inname en energie verbranding. Deze disbalans resulteert in extreme vetopslag van vooral het zogenaamde witte vet. Deze extreme vetopslag wordt ook wel adipositas of vetzucht genoemd. Adipositas heeft een negatieve invloed op de gezondheid en kan uiteindelijk bijdragen aan het ontwikkelen van ziekten, zoals bijvoorbeeld cardiovasculaire ziekten, type 2 diabetis mellitus of zelfs cognitieve achteruitgang en dementie. In het onderzoek beschreven in dit proefschrift hebben we onderzocht of de adipositas-brein-as beïnvloed kan worden door vetzuren in het dieet (arachidonzuur (ARA), docosahexaeenzuur (DHA) en butyraat) en daarnaast, of er in deze as een rol is weggelegd voor de hersendoorbloeding en adipokinen. De laatst genoemde, adipokinen zijn hormonen, eiwitten en cytokinen geproduceerd door het witte vetweefsel. Als laatste zullen we onderzoeken wat de invloed is van veroudering op de adipositas-brein-as.

Hoofdstuk 1 schetst de achtergrond van het onderzoek beschreven in dit proefschrift. We bespreken in dit hoofdstuk de rol van vetzuren in het ontstaan en tegengaan van adipositas. Zo is het bekend dat diëten met een hoge concentratie aan verzadigd vet en/of in combinatie met veel koolhydraten een belangrijke rol spelen in het veroorzaken van obesitas. Als tegengewicht, zijn er vetzuren die de ontwikkeling adipositas kunnen tegenwerken, voorbeelden zijn lange-keten-meervoudig-onverzadigde-vetzuren (LCPUFAs) en korte-keten-vetzuren (SCFAs, butyraat). De SCFAs kunnen worden gevormd door bacteriën in de darmwand wanneer deze bacteriën vezels verteren. Daaropvolgend bediscussiëren we via welke wegen adipositas de brein functie en cognitie zou kunnen beïnvloeden. Eén van de mogelijke wegen richting het brein zou kunnen zijn door het aantasten van de hersendoorbloeding, namelijk onder andere door verstijving en/of vernauwing van de vaten rondom het brein en ontstekingen in het brein. Deze veranderingen in de hersendoorbloeding kunnen daaropvolgend een negatieve invloed hebben op zenuwcellen in het brein, wat kan resulteren in cognitieve achteruitgang of zelfs dementie. Een andere weg richting het brein is vanuit het witte vetweefsel, zo zou de adipokinen productie verstoord kunnen zijn. Adipokinen zijn betrokken in vele biologische processen en kunnen ook op vele hun invloed uitoefenen. Als de productie van deze adipokinen verstoord is of uit balans doordat er extreem veel vetweefsel is, dan zouden zij neurodegeneratieve en vasculaire processen kunnen beïnvloeden wat uiteindelijk zou kunnen leiden tot cognitieve achteruitgang en dementie.

In het eerste experimentele onderzoek beschreven in **hoofdstuk 2**, onderzochten wij of de inname van de LCPUFAs, ARA en DHA in het vroege leven een beschermend effect kon hebben in muizen (ApoE3*Leiden). Deze muizen kregen drie weken na hun geboorte voor acht weken een dieet met de LCPUFAs, daaropvolgend kregen deze muizen voor 12 weken een hoog vet dieet (HFD). Om de effecten van deze diëten te onderzoeken hebben we een veelzijdige aanpak gebruikt om onder andere veranderingen in energie metabolisme, hersendoorbloeding, het brein en cognitie te kunnen analyseren. Uiteindelijk vonden wij dat een dieet met veel verzadigde vetzuren (hoog vet dieet; HFD) aanpassingen veroorzaakten op metabool vlak en in het brein, zoals bijvoorbeeld een toename van cholesterol en triglyceriden in het bloed, vermindering in verbindingen tussen hersengebieden en afname in grijze stof. Ook konden we concluderen dat de inname van ARA en DHA in het vroege leven bescherming biedt tegen de meeste veranderingen veroorzaakt door het HFD op latere leeftijd. Deze bevinding benadrukt de metabole flexibiliteit en cerebrale integriteit in het latere leven.

In **hoofdstuk 3** beschrijven we een onderzoek met als doel om het effect van korte-keten-vetzuur, butyraat te achterhalen in middelbare en oudere obese muizen. In dit onderzoek gebruikten we muizen die het gen voor de receptor van de lage-dichtheid-lipoproteïne missen, deze zogenaamde LDLr-/-.Leiden muizen. Deze muizen ontwikkelen op een HFD een zware vorm van obesitas. In dit onderzoek waren we met name geïnteresseerd in de ontwikkeling van adipositas, ontsteking van het witte vetweefsel, opslag van vet in de lever, bloeddruk en de cerebrale doorbloeding. We vonden dat een butyraat-interventie van twee maanden de negatieve effecten van een HFD voor een groot deel terug kon brengen naar normale controle waarden, en dit met name in de muizen van middelbare leeftijd. Daarbij ontdekten we dat een HFD de variatie verlaagt van bacteriën in de darm, en daarmee kan een HFD effect hebben op het verkrijgen van energie en vetopslag door bacteriën. Als laatste, vonden we dat butyraat de samenstelling van darmbacteriën kan veranderen, ondanks dat het lastig was om het effect van butyraat te onderscheiden van de effecten van een HFD op het darm bacteriën. We concludeerden uit dit onderzoek dat butyraat een belangrijke rol kan spelen in het tegenwerken van de ontwikkeling van adipositas op middelbare leeftijd.

Wij beschrijven in **hoofdstuk 4 en 5** onderzoeken uitgevoerd in mensen. In beide hoofdstukken hebben we met statische analysemodellen onderzocht of en hoe adipositas parameters zijn geassocieerd met het brein en dementie, om zo de adipositas-brein-as duidelijker te kunnen schetsen in mensen. De adipositas parameters die we analyseerden waren BMI, buikomvang, leptine en adiponectine. In **hoofdstuk 4** analyseerden we in de RUN DMC studie, body mass index (BMI),

buikomvang (WC), leptine en adiponectine (parameters voor adipositas), en in het brein bekeken we de afwijkingen aan de vaten, en grijze en witte stof volumes. De RUN DMC studie bestaat uit mannen en vrouwen die gediagnosticeerd zijn met ziekte aan de kleine vaten in het brein (CSVD). We vonden dat BMI, WC en leptine negatief geassocieerd zijn met volume van de grijze stof, met name in mannen (>50.0 jaar). Dit indiceert dat adipositas geassocieerd is met een lager grijze stof volume in mannen. Daarbij vonden we dat leptine een beschermend effect kan hebben in mannen, met betrekking tot de vasculaire parameters in het brein. In detail, leptine was negatief geassocieerd met verlies van integriteit van de wite stof (zogenaamde white matter hyperintensities) en de aanwezigheid van lacunes in het brein, welke beiden parameters zijn voor CSVD. Deze bevindingen indiceren dat parameters voor adipositas verschillend zijn geassocieerd met brein volume, vasculaire parameters, en geslacht. In **hoofdstuk 4** bediscussiëren we dat adipositas invloed kan hebben op hersenaandoeningen, zoals bijvoorbeeld verminderde grijze stof, en dat deze hersenaandoeningen zijn sterk geassocieerd met dementie.

In **hoofdstuk** 5 wordt een Zweeds onderzoek, de Gothenburg cohort studies beschreven. Deze studies bestaan uit Zweedse ouderen, man en vrouw, van 70 jaar en ouder. In deze studie analyseerden we of parameters voor adipositas (BMI, buik-heup-ratio, leptine en adiponectine) geassocieerd zijn met dementie over een periode van tien jaar. Daarbij analyseerden we ook of er een verschil was tussen mannen en vrouwen. Wij vonden dat in oudere vrouwen (>70.0 jaar), leptine en BMI verschillend geassocieerd zijn met het risico op dementia binnen 5-jaar. Dit terwijl je verwacht dat leptine en BMI dezelfde associatie hebben omdat ze sterk met elkaar gecorreleerd zijn. Zo is leptine is geassocieerd met een verminderde kans op dementie, terwijl een lage BMI (<20 kg/m^2) een verhoogd risico geeft om dementie te ontwikkelen binnen 5 jaar.

We bediscussiëren al onze resultaten in **hoofdstuk 6**, en daarnaast stellen we mogelijke andere toekomstige onderzoeken voor die vragen zou kunnen beantwoorden ontstaan uit het onderzoek beschreven in dit proefschrift. Ook bespreken we de belangrijkste conclusies van dit proefschrift, deze zijn: 1) er is een duidelijk verband tussen adipositas en brein functie. 2) Onderzoek in muismodellen biedt de kans om biologische processen en dieet interventies betrokken in de adipositas-brein-as in diepgaand detail te onderzoeken. 3) De inname van LCPUFAs in het vroege leven beschermt tegen adipositas, en behoudt van een gezonde brein functie. 4) Een butyraat interventie, en mogelijk de inname van vezels, kan de negatieve effecten van een HFD-dieet tegenwerken met name op middelbare leeftijd op het gebied van darm microbioom, hersendoorbloeding, lever en brein functie.

5) De adipositas-brein-as is een interactieve en multifactoriële as, die zeker wordt beïnvloed door geslacht en gedurende veroudering. Al met al, denken wij dat het onderzoek beschreven in dit proefschrift bijdraagt aan een verbeterd inzicht in de biologische mechanismen betrokken in de adipositas-brein-as. Uitgebalanceerde vezelrijke diëten met LCPUFAs kunnen een belangrijke rol spelen in het tegengaan van adipositas en de ontwikkeling van obesitas-gerelateerde-ziekten.

List of Abbreviations

AC	Auditory cortex
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's disease assessment scale-cognitive subscale
AgRP	Agouti-related peptide
ANOVA	Analysis of variance
APOC1	Apolipoprotein C-I
APOE	Apolipoprotein E
APOE3	Apolipoprotein E3
ApoE3*Leiden	Apolipoprotein E3 Leiden
APOEε4	Apolipoprotein E ε4 allele
ARA	Arachidonic acid
ASL	arterial spin labeling
BBB	Blood brain barrier
BMI	Body mass index
BOLD	Blood oxygen level-dependent
CA1	Cornu Ammonis area 1,
CART	cocaine and amphetamine-regulated transcript
CBF	Cerebral blood floow
cDNA	Complementary deoxyribonucleic acid
CM	Custom made
CNS	Central nervous system
CPRS	Comprehensive psychiatric rating scale
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CSVD	Cerebral small vessel disease
CT	Cycle threshold
CVD	Cardiovascular disease
DH	Dorsal hippocampus
DHA	Docosahexaenoic
DNA	Deoxyribonucleic acid
DSM-III-R	Diagnostic and statistical manual of mental disorders 3rd edition revised
DTI	Diffusion tensor imaging
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
FA	Fractional anisotropy
FAIR	Flow-sensitive alternating inversion recovery
FC	Functional connectivity

FFAR2	Free fatty acid receptor 2
FFAR3	Free fatty acid receptor 3
fMRI	Functional magnetic resonance imaging
FW	Forward
GAPDH	Glyceraldehydes 3-phosphate dehydrogenase
GLUT-1	Glucose transporter 1
GMV	Gray matter volume
H70	Gerontological and geriatric population studies
HDAC	Histone deacetylase
HDL	High density lipoprotein
HFD	High fat diet
HFDB	High fat diet enriched with butyrate
HFHC	High fat and high carbohydrates diet
HMW	High molecular weight
HV	Hippocampal volume
IBA-1	Ionized calcium binding adapter molecule-1
ICV	Intracranial volume
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
InsulinR	Insulin receptor
IVC	Individually ventilated cages
LCPUFAs	Long-chain polyunsaturated fatty acids
LDL	Low-density lipoprotein
LDL	Low density lipoprotein
LDLr	Low-density lipoprotein receptor
LDLr-/-	Low-density lipoprotein receptor knock-out Leiden
LTP	Long term potentiation
m.o.	Months old
MC	Motor cortex
MCP-1	Monocyte chemotactic protein-1
MD	Mean diffusivity
MED	Minimum entropy decomposition
MINI	Mini international neuropsychiatric interview
MMSE	Mini–Mental State Examination
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MWM	Morris water maze
n	Group size
NALFD	Non-alcoholic fatty liver disease
NaN3	Sodium azide
NE	North-East

NE	Not estimable
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
ObRA	Leptin receptor A
OBRB	Leptin receptor B
OF	Open field
OR	Odds ratio
ORT	Object recognition test
PAI-1	Plasminogen activator inhibitor
PBS	Phosphate-buffered saline
pCVD	Premature cardiovascular disease
PET	Positron emission tomography
PPARγ	Peroxisome profilerator-activated receptor-γ
PPSW	Prospective population study of women
PRIME	Preclinical imaging centre
PSD-95	Postsynaptic density protein-95
Q	Quartile
qRT-PCR	Quantitative real time polymerase chain reaction
REV	Reverse
RNA	Ribonucleic acid
ROI	Region of interest
rRNA	Ribosomal ribonucleic acid
rsfMRI	Resting state functional magnetic resonance imaging
RUN DMC	Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort
SAA	Serum amyloid A
SBP	Systolic blood pressure
SCFAs	Short chain fatty acids
SD	Standard deviations
SEM	Standard error of mean
SPF	Specific pathogen free
SSC	Somatosensory cortex
STRIVE	Strategies and therapies for reducing ischemic and vascular events
T2DM	type two diabetes mellitus
TBV	Total brain volume
TIA	Transient ischemic attack
TNF-α	Tumor necrosis factor alpha
TNO	Toegepast-natuurwetenschappelijk onderzoek
VC	Visual cortex
VCAM-1	Vascular cell adhesion molecule 1

VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A
VH	Ventral hippocampus
VLDL	Very low density lipoprotein
w/w	Weight by weight
WAT	White adipose tissue
WC	Waist circumference
WHR	Waist hip ratio
WMH	White matter hyperintensities
WML	White matter lesion
WMV	White matter volume
α-MSH	Alpha-melanocyte-stimulating hormone
β (95% C.I.)	Beta-coefficients and 95% confidence Intervals

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List of Publications

1. Kiliaan AJ, **Arnoldussen IAC**, Gustafson DR.
Adipokines: a link between obesity and dementia?
Lancet Neurology. 2014. (5-year impact factor: 28.0).

2. **Arnoldussen IAC**, Kiliaan AJ, Gustafson DR.
Obesity and dementia: adipokines interact with the brain.
European Neuropsychopharmacology. 2014. (5-year impact factor: 4.1).

3. **Arnoldussen IAC**, Kiliaan AJ.
Impact of DHA on metabolic diseases from womb to tomb.
Marine Drugs. 2014. (5-year impact factor: 4.2).

4. **Arnoldussen IAC**, Zerbi V, Wiesmann M, Noordman RH, Bolijn S, Mutsaers MP, Dederen PJ, Kleemann R, Kooistra T, van Tol EA, Gross G, Schoemaker MH, Heerschap A, Wielinga PY, Kiliaan AJ.
Early intake of long-chain polyunsaturated fatty acids preserves brain structure and function in diet-induced obesity.
The Journal of Nutritional Biochemistry. 2016. (5-year impact factor: 4.7).

5. **Arnoldussen IAC**, Wiesmann M, Pelgrim CE, Wielemaker EM, van Duyvenvoorde W, Amaral-Santos PL, Verschuren L, Keijser BJ, Heerschap A, Kleemann R, Wielinga PY, Kiliaan AJ.
Butyrate restores HFD-induced adaptations in brain function and metabolism in mid-adult obese mice.
International Journal Obesity. 2017. (5-year impact factor: 5.1).

6. **Arnoldussen IAC**, Sundh V, Bäckman K, Kern S, Östling S, Blennow K, Zetterberg H, Skoog I, Kiliaan AJ, Gustafson DR.
A 10-Year Follow-Up of Adiposity and Dementia in Swedish Adults Aged 70 Years and Older.
Journal of Alzheimers Disease. 2018. (5-year impact factor: 3.5).

7. **Arnoldussen IAC**, Leijsen EM, Gustafson DR, de Leeuw FE, Kiliaan AJ.
Body mass index is inversely associated with gray matter volumes. The RUN DMC study
Submitted to Neurology. 2018. (5-year impact factor: 8.3).

8. Pelgrim CE, Franx BAA, Snabel J, Kleemann R, **Arnoldussen IAC**, Kiliaan AJ.
Butyrate Reduces HFD-Induced Adipocyte Hypertrophy and Metabolic Risk Factors in Obese LDLr-/-.Leiden Mice.
Nutrients. 2017. (5-year impact factor: 4.6).

9. Franx BA, **Arnoldussen IAC**, Kiliaan AJ, Gustafson DR.
Weight Loss in Patients with Dementia: Considering the Potential Impact of Pharmacotherapy.
Drugs Aging. 2017. (5-year impact factor: 2.4).

10. van der Doelen RHA, Robroch B, **Arnoldussen IAC**, Schulpen M, Homb, Kozicz T.
Serotonin and urocortin 1 in the dorsal raphe and Edinger-Westphal nuclei after early life stress in serotonin transporter knockout rats.
Neuroscience. 2017. (5-year impact factor: 3.4).

11. van der Doelen RH, **Arnoldussen IAC**, Ghareh H, van Och L, Homberg JR, Kozicz T.
Early life adversity and serotonin transporter gene variation interact to affect DNA methylation of the corticotropin-releasing factor gene promoter region in the adult rat brain.
Development and Psychopathology. 2015. (5-year impact factor: 4.3).

12. Jansen D, Zerbi V, **Arnoldussen IAC**, Wiesmann M, Rijpma A, Fang XT, Dederen PJ, Mutsaers MP, Broersen LM, Lütjohann D, Miller M, Joosten LA, Heerschap A, Kiliaan AJ.
Effects of specific multi-nutrient enriched diets on cerebral metabolism, cognition and neuropathology in AβPPswe-PS1dE9 mice.
PLoS One. 2013. (5-year impact factor: 3.5).

13. Janssen CI, Zerbi V, Mutsaers MP, de Jong BS, Wiesmann M, **Arnoldussen IAC**, Geenen B, Heerschap A, Muskiet FA, Jouni ZE, van Tol EA, Gross G, Homberg JR, Berg BM, Kiliaan AJ.
Impact of dietary n-3 polyunsaturated fatty acids on cognition, motor skills and hippocampal neurogenesis in developing C57BL/6J mice.
The Journal of Nutritional Biochemistry. 2015. (5-year impact factor: 4.7).

14. Rijpma A, Jansen D, **Arnoldussen IAC**, Fang XT, Wiesmann M, Mutsaers MP, Dederen PJ, Janssen CI, Kiliaan AJ.
Sex Differences in Presynaptic Density and Neurogenesis in Middle-Aged ApoE4 and ApoE Knockout Mice.
Journal of Neurodegenerative Diseases. 2013. (5-year impact factor: 0.6).

15. Vreeken D, Wiesmann M, Deden LN, **Arnoldussen IAC**, Aarts E, Kessels, RPC, Kleemann R, Hazebroek EJ, Aarts EO, Kiliaan AJ.
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Sex specific alterations in brain structure, function and metabolism in juvenile HFD-induced obese LDLr-/- Leiden mice.
In preparation. 2018.

Curriculum Vitae

Ilse Arnoldussen werd geboren op 9 november 1988 te Gennep. Ze groeide op in Ottersum en behaalde haar VWO-diploma aan het Elzendaal college te Boxmeer in 2007. In datzelfde jaar begon zij aan de studie Biologie aan de Radboud Universiteit te Nijmegen. Vanwege haar grote interesse in het ontstaan van neurologische aandoeningen koos zij na het behalen van haar bachelordiploma voor een masteropleiding medische biologie met als focus neurowetenschappen.

Tijdens haar masteropleiding liep Ilse twee stages. Tijdens haar eerste masterstage onderzocht zij de rol van een multi-nutrienten dieet op het brein in muismodellen voor de ziekte van Alzheimer onder begeleiding van dr. Diane Jansen, dr. Valerio Zerbi en dr. Amanda J. Kiliaan. In haar tweede stage onderzocht Ilse de invloeden van stress in het vroege leven in ratten, waarbij zij vooral breinweefsel onderzocht op eiwit- en mRNA niveau. In deze tweede stage werd zij begeleid door dr. Rick van der Doelen en dr. Tamás Kozicz. Beide stages vergrootte haar interesse in neurowetenschappen en voeding, daarnaast haar voorliefde om *in vivo* experimenten op te volgen tot in groot detail in *ex vivo* hersenweefsel.

In oktober 2012 begon Ilse haar promotieonderzoek op de afdeling Anatomie van het Radboudumc te Nijmegen onder begeleiding van copromotoren: dr. Amanda J. Kiliaan en dr. Maximilian Wiesmann, en promotoren: prof. dr. Deborah R. Gustafson en dr. Otto Boerman. Tijdens haar promotie beschreven in dit proefschrift voerde zij een aantal studies uit in muismodellen in samenwerking met Mead Johnson (RB) en TNO Leiden. In de tweede fase van haar promotieonderzoek reisde zij naar New York city om onder begeleiding van haar promotor, Professor Gustafson zich verder te verdiepen in epidemiologisch onderzoek. Tijdens haar promotietraject begeleidde zij een tiental studenten gedurende hun stages. Tevens presenteerde zij haar bevindingen op verschillende nationale en internationale bijeenkomsten in de vorm van posters en mondelinge presentaties. Heden is Ilse werkzaam als Post-doc op het EFRO BriteN project aan de afdeling Anatomie, Radboudumc te Nijmegen.

Radboud Alzheimer Centrum Series

1. Van Horssen, J. (2005). *Heparan sulfate proteoglycans and vascular pathology in Alzheimer's disease*
2. Wilhelmus, M.M.M. (2006). *Small heat shock proteins and apolipoprotein E in Alzheimer's disease*
3. Zuidema, S.U. (2008). *Neuropsychiatric symptoms in Dutch nursing home patients with dementia*
4. Graff, M.J.L. (2008). *Effectiveness and efficiency of community occupational therapy for older people with dementia and the caregivers*
5. Claassen, J.A.H.R. (2008). *Cerebral hemodynamics in aging: the interplay between blood pressure, cerebral perfusion, and dementia*
6. Dado- Van Beek, H.E.A. (2010). *The regulation of cerebral perfusion in patients with Alzheimer's disease*
7. De Jong, D. (2010). *Anti-inflammatory therapy and cerebrospinal fluid diagnosis in Alzheimer's disease*
8. Persoon, J.W.B. (2010). *Development and validation of the Nurses' Observation Scale for Cognitive Abilities – NOSCA*
9. Meulenbroek, O.V. (2010). *Neural correlates of episodic memory in healthy aging and Alzheimer's disease*
10. Timmer, N.M. (2011). *The interaction of heparan sulfate proteoglycans with the amyloid b protein*
11. Schölzel-Dorenbos, C.J.M. (2011). *Quality of life in dementia: From concept to practice*
12. Bruinsma, I.B. (2011). *Amyloidogenic proteins in Alzheimer's disease and Parkinson's disease: interaction with chaperones and inflammation*
13. Perry, M. (2011). *Development and evaluation of a Dementia Training Programme for Primary care*
14. Derksen, E.W.C. (2011). *Diagnostic disclosure: a basic intervention in dementia care*
15. Wetzels, R.B. (2011). *Neuropsychiatric symptoms in institutionalized residents with dementia: Course and interplay with cognition,quality of life and psychotropic drug use.*
16. Voight-Radloff, S. (2012). *Cross-national transfer of community occupational therapy in dementia.*

17. Spies, P.E. (2012). *The reflection of Alzheimer disease in CSF.*

18. Joosten-Weyn Banning, E.W.A. (2012). *Learning to live with Mild Cognitive Impairment: development and evaluation of a psychological intervention for patients with Mild Cognitive Impairment and their significant others.*

19. Vasse, E. (2012). *A stepwise process for developing and implementing quality indicators to improve psychosocial dementia care in European countries.*

20. Slats, D. (2012). *CSF biomarkers of Alzheimer's disease; serial sampling analysis and the study of circadian rhythmicity.*

21. Leontjevas, R. (2012). *Act in case of Depression! Validation and effectiveness of a multidisciplinary depression care program in nursing homes.*

22. Bakker, C. (2013). *Young onset dementia: care needs & service provision.*

23. Meeuwsen, E.J. (2013). *Towards efficient dementia care : a comparison of memory clinics and general practitioners.*

24. Spijker, G.J.A.A (2013). *Systematic care for caregivers of people with dementia in community mental health services.*

25. Janssen, D. (2013). *The role of nutrition in Alzheimer's disease : a study in transgenic mouse models for Alzheimer's disease and vascular disorders.*

26. Zerbi, V. (2013). *Impact of nutrition on brain structure and function : a magnetic resonance imaging approach in Alzheimer mouse models.*

27. Herbert, M. (2014). *Facing uncertain diagnosis: the use of CSF biomarkers for the differential diagnosis of neurodegenerative diseases.*

28. Ven, G. van de (2014). *Effectiveness and costs of Dementia Care Mapping intervention in Dutch nursing homes*

29. Döpp, C.M.E. (2015). *Making the jump-The translation of research evidence into clinical occupational therapy practice.*

30. Elsen, G. van den (2016). *Tetrahydrocannabinol in the treatment of neuropsychiatric symptoms in dementia.*

31. Vermeij, A. (2016). *Cognitive plasticity in normal aging and mild cognitive impairment: Shedding light on prefrontal activation.*

32. Müller, M. (2016). *Footprints of Alzheimer's disease. Exploring proteins and microRNAs as biomarkers for differential diagnosis.*

33. Bruggink, K.A. (2016). *Amyloid-β and amyloid associated proteins in the pathology and diagnosis of Alzheimer's disease.*

34. Aalbers, T. (2016). *eHealth in the primary prevention of cognitive decline; The Brain Aging Monitor study.*

35. Van der Maaden, T. (2016). *Improving discomfort in nursing home residents with dementia and pneumonia. Development, implementation and evaluation of a practice guideline for optimal symptom relief.*

36. Millenaar, J. (2016). *Young onset dementia, towards a better understanding of care needs and experiences.*

37. Rijpma, A. (2017). *Multi-nutrient interventions and brain metabolism in Alzheimer's disease: a spectrum of effects.*

38. Wiesmann, M. (2017). *Vascular risk factors and Alzheimer's disease. Therapeutic approaches in mouse models.*

39. Jong, D.L.K. de (2018). *Regulation of cerebral perfusion in Alzheimer's disease: from seconds to months.*

40. Donkers, H.W. (2018). *Social Participation Dilemma's in dementia.*

41. Richters, A. (2018). *Network-based care for people with dementia: a complex transition.*

42. Spek, K. van der (2018). *Appropriate psychotropic drug use in institutionalized people with dementia. The PROPER-study.*

43. Arnoldussen, I.A.C. (2018). *Adiposity and the Brain.*

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrolment of the best and most motivated students.

The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc.. Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy.

For more information on the DGCN as well as past and upcoming defenses please visit:

http://www.ru.nl/donders/graduate-school/phd/